COMPARATIVE EVALUATION OF THE ANTIMICROBIAL EFFICACY OF THREE DIFFERENT CHEMICAL DISINFECTANTS AND THEIR EFFECT ON THE DIMENSIONAL STABILITY OF POLYVINYL SILOXANE (PVS) IMPRESSIONS

Dissertation Submitted to

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH I

PROSTHODONTICS AND CROWN & BRIDGE

APRIL 2011

CERTIFICATE

This is to certify that the dissertation titled "**COMPARATIVE EVALUATION OF THE ANTIMICROBIAL EFFICACY OF THREE DIFFERENT CHEMICAL DISINFECTANTS AND THEIR EFFECT ON THE DIMENSIONAL STABILITY OF POLYVINYL SILOXANE (PVS) IMPRESSIONS**" is a bonafide record work done by **Dr.J.VIDHYA** under our guidance and to our satisfaction during her post graduate study period between 2008 – 2011.

This Dissertation is submitted to **THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY**, in partial fulfillment for the Degree of **MASTER OF DENTAL SURGERY – PROSTHODONTICS AND CROWN & BRIDGE, BRANCH I.** It has not been submitted (partial or full) for the award of any other degree or diploma.

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ACKNOWLEDGEMENT

*First of all, I would like to thank **Almighty God** for giving me the strength, courage and confidence to overcome all hurdles and finish this arduous job.*

This dissertation is the result of work with immense support from many people and it is my pleasure to take this opportunity to express my gratitude to all of them.

*I would be failing in my duty if I do not adequately express my deep sense of gratitude and my sincere thanks to **Dr. N. S. Azhagarasan M.D.S., Professor, Head of the Department, Department of Prosthodontics and Crown & Bridge**, Ragas Dental College and Hospital, Chennai, for his exceptional guidance, tremendous encouragement and well timed suggestions throughout my postgraduate programme. I would like to profoundly thank him for giving an ultimate sculpt to this study.*

*I wish to express my gratitude to **Dr. S Ramachandran M.D.S., Principal,** Ragas Dental College, Chennai for his encouragement and support throughout my post graduate course. I also thank him for permitting me to make use of the amenities in the institution.*

*I would like to express my real sense of respect, gratitude and thanks to my **Guide, Dr. K. Chitra Shankar M.D.S**., **Professor** for her guidance, constant support and encouragement extended to me during this study. Her patience and perseverance benefited me in every facet of my study and I thoroughly enjoyed every discussion with her. Her valuable guidance and positive criticisms enabled*

me to develop an understanding of the subject from the concept to the conclusion. Her guidance and supervision helped me to bring the best out of me in this study. The timely help and encouragement rendered by her had been enormously helpful throughout my study.

I would also like to thank ***Dr. K. Madhusudan M.D.S., Dr. S. Jayakrishnakumar, M.D.S., Dr. Manoj Rajan M.D.S. and Dr. Saket Miglani M.D.S.,*** *for their valuable suggestions and support given throughout my study.*

My sincere thanks to ***Dr. Manikandan, M.D.S., Dr. Hariharan, M.D.S., Dr. Saravana Kumar, M.D.S.,*** *and* ***Dr. Vallabh Mahadevan, M.D.S.,*** *who supported me during this study.*

I express my sincere thanks to ***Dr. Usha Ananth Rao****,* ***Director Research and Professor*** *, Department of Microbiology, Ragas Dental College and Hospital, Chennai for extending her valuable support and expertise in the microbiological phase of this study. Her easy approachability laced with constant encouragement helped me to complete this aspect of the study.*

I wish to convey my sincere thanks to ***Dr. B. Vikraman M.D.S****.,* ***Professor,*** *In-charge of Virtual Lab and CT data conversion and simulation, Department of Oral and Maxillofacial Surgery, Ragas Dental College and Hospital, Chennai for permitting me to use 3D image processing software (MIMICS), which was a novel method used in the study for linear measurements. I owe an enormous debt of gratitude and thanks to him for his insightful guidance and willingness to share*

his limitless knowledge in taking up this novel method for measurement in this study.

I also would like to make a special reference to ***Mr. Velappan Jeyakumar****,* ***Managing Director, M/s. Tiaano Ti Anode Fabricators Private Limited, Chennai*** *for providing me with freshly prepared Electrolyzed Oxidizing Water (EOW) for this study. His valuable guidance provided me enormous confidence to test EOW as a dental disinfectant.*

I would like to convey my gratitude to ***Mr. Ashok kumar, Technician, M/s. Arthi Scans****, Chennai, for helping me to complete the scanning of the impressions, which is an essential step in this study.*

My thanks to ***Dr.R.Ravanan****, Reader, Department of Statistics, Presidency College, Chennai, for his valuable help with the statistical work for this study.*

It would not be justifiable on my part if I do not acknowledge the help of my fellow colleagues, my seniors, juniors and friends for their constant encouragement and continued support throughout my post graduate course. I thank all my patients who consented and participated in this study.

Last but not the least, even though words would not do much justice, I would like to specially thank my husband and parents for their blessings, love and support.

INTRODUCTION

Dental impressions are the stepping stones for fabrication of removable and fixed prostheses. It has been documented that impressions can be contaminated with pathogenic agents that are absorbed and adsorbed on them. Thus the risk of contamination from patients to clinical and laboratory personnel exists. Casts produced from contaminated impressions may themselves be contaminated, because microorganisms are able to migrate from the impressions into the casts. [21, 33, 35]

Various methods for disinfecting and sterilizing different impression materials have been documented. Disinfection can be done by immersion or spraying of various chemical disinfectants, while sterilization measures include exposure to ethylene oxide gas, microwave, ultraviolet light and autoclaving. [2,4,7,16,20,22,24,29,46] However, no single method has been able to fulfill all disinfection requirements. For example, immersion in chemical solutions provide satisfactory antimicrobial efficacy but can compromise the dimensional accuracy of hydrocolloid impressions; exposure to ultraviolet light does not produce a satisfactory antibacterial effect; and microwave is not suitable for impressions on metal trays. Similarly ethylene oxide gas sterilization for dental impressions is not a feasible option for all clinical settings. Autoclaving of impressions may eliminate all microbial contamination including spores, but is time consuming and not suitable for all impression materials[63]. Thus, disinfection rather than sterilization of dental impressions is a more practical approach on a day-today basis.

Disinfection can be accomplished by using chemicals by immersion or spraying. Studies have revealed that immersion disinfection exposes all the impression surfaces more favorably to the disinfectant compared to spraying. However, immersion is also known to cause greater dimensional and surface changes of an impression as compared to spraying.[4,25,38,43]

Polyvinyl Siloxane (PVS) impression materials are widely used in the field of Prosthodontics due to their excellent overall physical properties and good patient acceptance.[11,13,42] Glutaraldehyde and sodium hypochlorite have been recommended as disinfectants for PVS impressions.[6,12,37,44] Glutaraldehyde, a "high-level disinfectant", is a bactericidal, virucidal and fungicidal that is an effective disinfectant for silicone impressions.[10,16] Sodium hypochlorite, in a concentration of 1% has been reported as an "intermediate-level disinfectant".[16, 17, 58]

The commonly isolated oral pathogens from in-vivo impressions include Streptococcus, MRSA, Candida albicans, Pseudomonas aeruginosa, E-coli and M. Tuberculosis.[8,10,15,16,48,51] Immersion of PVS impressions in 2% glutaraldehyde and 1 % sodium hypochlorite has resulted in successful disinfection as revealed in previous studies.[3,5,7,10,16,36,63]

Most of the researches done are in-vitro studies, where the effects of disinfectants are noted on artificially contaminated impressions. It has been reported that in-vitro studies have limitations and may not exactly simulate clinical (in-vivo) evaluation. Clinical studies evaluating the antimicrobial efficacy of immersion disinfectants on patient derived PVS impressions are very few.[7,10,15,16]

Recent research in Japan has revealed that freshly prepared Electrolyzed Oxidizing Water (EOW) has strong microbicidal properties. EOW has been widely used in the food, medical, veterinary medicine and poultry industries as a disinfectant agent. EOW as a disinfectant was investigated by Wu G., et al in a recent study on hydrocolloid impressions, gypsum casts and titanium samples. Their results showed an adequate antimicrobial efficacy of EOW for the above materials. However, the effect of immersion in EOW in disinfecting patient - derived PVS impressions has not been investigated.[19,45,61,63]

The dimensional stability of an impression material reflects its ability to maintain the accuracy of the impression over time. Obtaining an undistorted impression after disinfection is critical to the fit of the future prostheses. Hence disinfection procedures that provide adequate antimicrobial efficacy without affecting the changes in impression dimensions are the focus of researchers.[31] Various methods such as, Boley's gauge, measuring microscope, digimatic calipers, Nikon measurescope, have been used to measure the dimensional changes of impressions directly or indirectly.[3,5,14,24,25,30,36,39,56-59,62] More sophisticated techniques such as evaluation of CT scan overlays have been recently described for PVS impressions. Linear measurements done on images obtained by CT and reconstructed using appropriate software such as Mimics have been employed with acceptable results for measuring images of orthodontic casts and anthropometric measurements. Linear measurements done on CT-reconstructed 3D images have been considered to be accurate.[8, 29, 47]

PVS impression material seems to be relatively unaffected dimensionally by immersion in disinfectants such as glutaraldehyde and sodium

hypochlorite.[5,14,23,25,33,36] Most of these studies have been done on test dies[2,5,24,32,38,49] or casts[1,3,25,36,52] obtained from the impressions. Data on dimensional changes obtained by direct evaluation of PVS impression is sparse. In a previous study, a ten minute immersion in EOW caused significant dimensional changes in hydrocolloid impressions.[63] The effect of immersion in EOW on dimensional stability of PVS impressions has not been studied.

In light of the above, the aim of the present study was to comparatively evaluate the antimicrobial efficacy of three different chemical disinfectants and their effect on the dimensional stability of polyvinyl siloxane (PVS) impressions. Also glued to the aim were the following objectives:

1. To quantitatively and qualitatively evaluate the presence and type of oral microbial flora by microbial culture of Untreated (Control) patient-derived PVS impressions **(Group-I)**.
2. To quantitatively and qualitatively evaluate the presence and type of oral microbial flora by microbial culture of patient-derived PVS impressions subjected to immersion in commercially available 2.4% Glutaraldehyde **(Group-II).**
3. To quantitatively and qualitatively evaluate the presence and type of oral microbial flora by microbial culture of patient-derived PVS impressions subjected to immersion in commercially available 1 % Sodium hypochlorite **(Group-III)**.
4. To quantitatively and qualitatively evaluate the presence and type of oral microbial flora by microbial culture of patient-derived PVS impressions

subjected to immersion in freshly prepared Electrolyzed Oxidizing Water (EOW) **(Group-IV).**

5. To quantitatively compare the antimicrobial efficacy of the above three chemical disinfectants with respect to the control group and to each other on patient-derived PVS impressions.
6. To evaluate the dimensional stability of dental model-derived PVS impressions immersed in 2.4% Glutaraldehyde by Computed Axial Tomography (CAT) scanning and 3D reconstruction using Mimics software **(Group-V).**
7. To evaluate the dimensional stability of dental model-derived PVS impressions immersed in 1 % Sodium hypochlorite by Computed Axial Tomography (CAT) scanning and 3D reconstruction using Mimics software. **(Group-VI).**
8. To evaluate the dimensional stability of dental model-derived PVS impressions immersed in freshly prepared Electrolyzed Oxidizing Water (EOW) by Computed Axial Tomography (CAT) scanning and 3D reconstruction using Mimics software **(Group-VII).**
9. To compare the dimensional stability of dental model-derived PVS impressions subjected to immersion in three different chemical disinfectants within the three groups **(Groups V, VI, & VII).**
10. To compare the differences in dimensional changes of dental model-derived PVS impressions subjected to immersion in three different chemical disinfectants between the three groups **(Groups V, VI, & VII).**

REVIEW OF LITERATURE

Leung RL et al (1983)[33] studied microbial contamination on gypsum casts by incubating with brain heart infusion medium (BHI) and found microbial growth indicating cross contamination.

Valderhaug J et al (1984)[60] compared the dimensional stability of impressions made with polyether and silicone on metallic master models of the upper jaw. The canines and first molars represented abutment teeth with flat occlusal surface. The impressions were assessed directly to avoid loss of details due to cast pouring by measuring between these reference points.

Johansen RE et al (1987)[24] evaluated the linear dimensional changes of different rubber elastomers after immersion in 2% activated glutaraldehyde solution. Polyvinyl siloxane impressions demonstrated greater stability.

Drennon DG et al (1989)[14] examined five disinfectants applied by spray atomization for dimensional distortion and anti microbial efficacy on polyether, polysulfide, and addition silicone impressions and type IV gypsum casts. The disinfectants did not affect dimensional stability significantly with acceptable efficacy. The most accurate cast was produced by addition silicone impressions.

Jones ML et al (1989)[26] assessed the dimensional stability of various disinfection techniques on alginate impressions. Linear measurements of the resultant study casts were made using both canines and first molars.

Langenwalter EM et al (1990)[32] evaluated the distortion caused by disinfection by immersion on polysulfide, polyether and vinyl siloxane impressions as per ADA specification No.19 with iodophor 0.0075%, sodium

hypochlorite 0.05%, glutaraldehyde 2% and double deionized water. Linear measurements taken with a travelling microscope gave insignificant changes.

Matyas J et al (1990)[36] studied dimensional changes of disinfected impression materials on full arches and dies with the measuring microscope. Full arch measurements were done in anterior and posterior segments using canines and molars as references.

Samaranayake LP et al (1991)[54] assessed the carriage and persistence of oral flora on irreversible hydrocolloid and elastomeric impression materials. Retention of bacteria was greater on irreversible hydrocolloids compared with elastomers and significantly greater microbial load on dentate impressions.

Shillingburg HT et al (1997)[55] stated that impression must be handled properly and an accurate impression can be distorted by improper handling. An accurate undistorted impression is a must for well fitting restorations.

Johnson GH et al (1998)[25] concluded that dimensional accuracy of gypsum cast and dies retrieved from disinfected addition silicone impressions assessed by means of measuring microscope to be most accurate.

McCabe JF (1998)[37] stated that a standard disinfection regime of 10 minute immersion in sodium hypochlorite and a prolonged immersion in glutaraldehyde solution will have no effect on the dimensional stability of addition curing silicone impression materials.

Adabo GL et al (1999)[3] studied effect of disinfection methods on the dimensional stability of six elastomeric materials by immersing in 5.25% sodium hypochlorite solution for 10 minutes, and 2% glutaraldehyde solution for 30

minutes. Measurements were done using the cusp tip of the left canine and the cusp tip of the distobuccal cusp of the left first maxillary molar as reference.

Venkitanarayanan KS et al (1999)[61] described and studied the efficacy of electrolyzed oxidizing water (EOW) for inactivating Escherichia coli, Salmonella enteritidis, and Listeria monocytogenes. An exposure time of 5 minutes reduced the populations of all three pathogens in the treatment samples by approximately 7 log CFU/ml, with complete inactivation by 10 min of exposure. Results indicated that electrolyzed oxidizing water may be a useful disinfectant.

Ivanis T et al (2000)[23] studied dimensional stability of elastomeric materials after disinfection and found the least changes in addition silicone.

Kugel G et al (2000)[31]surveyed disinfection practices in U.S. dental laboratories and documented that majority of impressions were polyvinyl siloxane and suggested disinfection times in accordance with ADA recommendations.

Buck JW et al (2002)[9] studied potential of Electrolyzed Oxidizing Water (EOW) to control foliar diseases in green houses. EOW reduced or eliminated germination of all 22 fungi species. They highlighted that EOW is safe to handle and several hospitals in Japan use EOW for surface sterilizing and hand washing.

Georgescu CE et al (2002)[21] analyzed the potential ways of cross contamination in dental practice and highlighted the universal rules for infection control. Transmission of infection occurs through impressions that are a potential vehicle in transmission of infectious agents resulting in contaminated casts.

O Brien WJ (2002)[44] recommended disinfection of addition silicone impressions by immersion in sodium hypochlorite, iodophors, complex phenolics,

glutaraldehydes, or phenolic glutaraldehydes.

Fabrizio KA et al (2003)[18] monitored the oxidation reduction potential (ORP), chlorine concentrations and pHs of acidic and basic Electrolyzed Oxidizing Water (EOW) for 3 days at 4° C and 25°C and concluded that the free chlorine concentration of acidic EOW stored at 25° C decreased after 24 hours.

Abdelaziz KM et al (2004)[1] studied dimensional changes on disinfected stone casts by linear measurements between canines and molars using digital micrometer.

Abdelaziz KM et al (2004)[2] evaluated the dimensional accuracy and wettability of vinyl polysiloxane and polyether impressions after sterilization by immersion in 2% glutaraldehyde, autoclaving and microwave radiation and concluded that sterilization with latter methods resulted in some dimensional change and topical surfactant was needed to improve the wettability.

Donavan TE et al (2004)[13] reviewed the ideal properties of impression materials with special emphasis on polyvinyl siloxane (PVS) impression materials. They stated that PVS materials have the best fine detail reproduction and elastic recovery of all available materials, possess remarkable dimensional stability and so can be used in a variety of clinical situations.

Junevicius J et al (2004)[27] showed that infection can be transmitted through insufficiently decontaminated alginate and silicone impressions. Using serial dilution method of counting they concluded that the silicone impressions get less contaminated with microorganisms than alginate impressions.

Muller-Bolla M et al (2004)[43] surveyed disinfection protocols in European dental schools and found wide variations. They stated that spraying of disinfectant may be an inadequate procedure and called for further research to develop universal disinfection guidelines.

Anusavice KJ (2005)[6] recommended disinfection of addition silicones with all EPA registered disinfectants without adverse dimensional changes provided the disinfection time is short such as glutaraldehydes and chlorine compounds for silicones.

Paola CL et al (2005)[45] concluded that an exposure time of five minutes to EOW gave log reduction of microbial population on lettuce by 6.6**.**

Fenner DC et al (2006) [19] widely evaluated antimicrobial efficacy of anodic electrolyzed oxidizing water (EOW) and found strong bacterial against Gram positive, Gram negative bacteria as well as C.albicans in 5 min exposure to 5 % EOW (20 mg/lit Cl_2). Large amount of HOCl on account of low pH of 3 is considered the chief factor of disinfecting efficacy of EOW.

Al-Jabrah O et al (2007)[4] recommended testing antimicrobial efficacy on patient-derived impressions and studied the effectiveness of four different disinfectant solutions on alginate, polyether, and polyvinyl siloxane. Clinical impressions were obtained, exposed to six different regimens and cultured. Serial dilution method for colony counting was followed. The results showed that all disinfectants were able to completely eliminate microorganisms carried by the impressions. Among undisinfected specimens, untreated alginate impressions carried more microorganisms than the rubber impression materials.

Martin N et al (2007)[34] examined the effect of several disinfectant systems upon the dimensional stability of alginate, addition cured silicone, condensation cured silicone and polyether impressions by direct measurement with a custom built automatic laser micrometer without pouring a cast.

Egusa H et al (2008)[15] assessed the persistent presence of microorganism on patient-derived impressions and gypsum casts. As a negative control, a maxillary arch of a sterilized, standard typodont with rubber-simulated soft tissue was used. Brain Heart Infusion (BHI) agar medium was used for microbial culture. The isolated pathogens include Candida, MRSA and P aeruginosa.

Egusa H et al (2008)[16] clinically evaluated the disinfection efficacy of commercially available disinfectants including 2% glutaraldehyde, 1% sodium hypochlorite, in removing oral pathogens from patient - derived alginate impressions. Brain Heart Infusion (BHI) agar medium was used for microbial culture. The isolation frequencies of streptococci, staphylococci, Candida, methicillin-resistant Staphylococcus aureus, and Pseudomonas aeruginosa species from undisinfected impressions were 100%, 55.6%, 25.9%, 25.9% and 5.6%, respectively. Potential bacterial contamination could be detected even after immersion for 10 minutes in 2% Glutaraldehyde and 1 % Sodium hypochlorite.

Melilli D et al (2008)[38] studied the effect of immersion disinfection procedures on the dimensional stability of a polyether and an addition silicone. Disinfection by immersion was recognized as more effective and reliable than disinfection by spray, as the disinfectant solution comes into contact with all the surfaces of the impression material by immersion with insignificant changes.

Wu G et al (2008)[63] evaluated the feasibility of using Ultrasonically Nebulised Electrolyzed Oxidizing Water (UNEOW) as a new universal approach for disinfecting irreversible hydrocolloid impressions, dental metals and gypsum casts with high bactericidal efficacy but without affecting dimensional accuracy and surface quality. The impressions were subjected to disinfection by (1) immersion in 1% sodium hypochlorite for 10 minutes; (2) immersion in EOW for 10 min; (3) exposure to UNEOW for 15, 30 and 45 minutes; (4) no disinfection (control). Dimensional accuracy, surface quality, and effect of corrosion were evaluated. Results showed that immersion in EOW resulted in a 100% kill rate and $\log_{10}$ reduction greater than six for S. aureus and B. subtilis. Immersion in Sodium hypochlorite resulted in $\log_{10}$ reduction around 4, which is the gold standard for a dental disinfectant. The kill rates of sodium hypochlorite were all a little lower than 30 min UNEOW treatment. The 10 min immersion in EOW and 1% sodium hypochlorite resulted in significant dimensional changes in alginate samples.

Amin WM et al (2009)[5] evaluated the effect of disinfecting impression materials on the dimensional accuracy and surface quality of the resulting casts on steel die constructed according to ANSI/ADA specification No.18 & 19 and tested alginate, addition silicone, condensation silicone and zinc oxide eugenol paste with different agents. Dimensions of the disinfected impressions and their resultant casts were measured using a computerized digital caliper. Addition silicone showed the best surface quality and dimensional stability.

Atabek D et al (2009)[7] investigated effects of disinfection agents on impression materials in-vivo. Dental impressions were taken from each patient

with an irreversible hydrocolloid impression material and control impressions were rinsed with 250 cc distilled water without any disinfecting procedure. Impressions were disinfected with 7.5 % povidone iodine solution for 3 minutes, 1 % sodium hypochlorite solution for 3 minutes and 1 % sodium hypochlorite solution for 10 minutes. Microorganisms were found in control samples and not when 7.5 % Povidone Iodine or 1 % Sodium Hypochlorite was used.

Baumgaertel S et al (2009)[8] investigated the reliability and accuracy of dental measurements made on cone-beam computed tomography (CBCT) reconstructions. Human skulls were scanned with dental CBCT, and 3D reconstructions of the dentitions were generated. Measurements were made directly on the dentitions of the skulls with a high-precision digital caliper and on the digital reconstructions with commercially available software. CT measurements showed high reliability.

Giammanco GM et al (2009)[22] evaluated the ability to resist disinfection by dental impressions obtained with a polyether and an addition polymerized silicone. An artificial dental arch was used as a model for the impressions, which were contaminated with a mixture of three biofilm-forming microorganisms. Two disinfectants with and without glutraldehyde were tested and found to be effective in reducing the microbial presence on the impression materials.

Bustos J et al (2010)[10] conducted a dual study of determining the effectiveness of disinfection with 0.5% NaOCl and 2% glutaraldehyde solutions on irreversible hydrocolloid and silicone impressions and analyzing structural changes at the surface level. Impressions were taken from maxillary dentate patients. They concluded that immersion in 0.5% NaOCl and 2% glutaraldehyde

for 10 minutes completely eliminated bacteria in the impressions, compared with the control group.

Kamagawa M et al (2010)[28] demonstrated direct three-dimensional impression modeling using microfocus X-ray CT to eliminate the conversion process to dental casts and found CT images to be acceptable.

Kollefrath R et al (2010)[29] conducted a study to demonstrate the clinical feasibility of autoclaving silicone impression materials. Two impressions were made of fixed partial denture preparations on the same patient using polyvinyl siloxane (PVS) impression material. The second impression was disinfected, subjected to a computer tomography (CT) scan, autoclaved and then subjected to a second CT scan. The CT overlays demonstrated the dimensional changes for the second impression after autoclaving to be acceptable. Both impressions were sent for restoration fabrication. The dimensions of the final restorations made from autoclaved impressions did not differ from those made from conventionally disinfected impressions.

Marya CM et al (2010)[35] confirmed that the dental profession is at three times the risk of contacting diseases and recommended appropriate disinfection of impressions.

Pichler W et al (2010)[47] conducted an anatomical study to measure the scaphoid using a 64-slice SOMATOM Sensation CT system (resolution 0.6 mm). Three-dimensional reconstructions from the raw data were generated by MIMICS software (Materialise, Leuven, Belgium) and the linear distances from different points were measured. They concluded that computerized analysis may result in a significant reduction of measurement errors.

MATERIALS AND METHODS

The present study was conducted to comparatively evaluate the antimicrobial efficacy of three different chemical disinfectants and their effect on the dimensional stability of polyvinyl siloxane (PVS) impressions.

The following Materials and Equipments were used for the study:

MATERIALS EMPLOYED:

- Polyvinyl Siloxane (PVS) impression Material – Addition type (Affinis- Coltene Whaledent, Germany)
    - Putty super-soft consistency(Fig.1a)
    - Light body consistency(Fig.1b)
    - Auto mixing gun (JSP Dental, California, USA) (Fig.1c)
    - Mixing tip (Affinis- Coltene Whaledent, Germany) (Fig.1d)
    - Proportioning scoops (Affinis- Coltene Whaledent, Germany) (Fig 1e)
- Maxillary model with typodont teeth (Nissin Dental Products Inc, Japan) (Fig.2)
- Metallic impression trays (Jabbar & Co, India) (Fig.4)
- Nitrile gloves, (Kimberly clark hygiene products Pvt. Ltd, Pune, India.) (Fig.5)
- Polypropylene container (Parsons Pvt. Ltd Mumbai, India) (Fig. 6)
- 2.4 % Glutaraldehyde solution (Cidex, Johnson & Johnson Ltd, India) (Fig.7)
- Cotton roll (Ramraju Surgical Cotton Mills Ltd, Rajalaiyam,India) (Fig. 8)
- Distilled water (Vijayshree traders, Chennai) (Fig.11a)

- Calibrated beaker (Borosil Glass Works Ltd, Ahmedabad, India) (Fig. 11 b)
- Disposable drape (Plasti Surge Industries Pvt. Ltd, Amravati, India) (Fig.14 a)
- Disposable head cap (Crosscare Surgical Disposables, Chennai, India) (Fig. 14 b)
- Disposable face mask (Crosscare Surgical Disposables, Chennai, India) (Fig.14 c)
- 1 % Sodium hypochlorite solution (Alan Medical products, Chennai, India) (Fig.19a)
- Freshly prepared Electrolyzed Oxidizing Water (EOW) with 50 mg/l free chlorine, with pH of 2.5, and ORP of 1150mv (Tianno Ti Anode Fabricators Pvt Ltd, Chennai, India) (Fig.20a)
- Saline (Nirlife Healthcare Nirma Limited, Gujarat, India)(Fig.22a)
- Calibrated cylinder (Borosil Glass Works Ltd, Ahmedabad, India) (Fig. 22b)
- Micro pipette (Accumax, fine care bio systems, Gujarat,India) (Fig. 23a)
- Calibrated wire loop (Himedia Laboratories Ltd, Mumbai, India) (Fig.23b)
- Brain Heart Infusion agar (BHI) (Himedia laboratories Ltd, India) (Fig.24a)
- Culture plate (Borosil Glass Works Ltd, Ahmedabad, India) (Fig.24 b)
- Glass marking pencil (Hindustan pencils ltd, Mumbai) , (Fig 25 a)
- Test tubes in test tube stand (Borosil Glass Works Ltd, Ahmedabad, India) (Fig. 28)
- Sabouraud agar (Himedia Laboratories Ltd, Mumbai, India) (Fig.32)

- Blood agar (BHI custom-made with 2% defibrinated blood) (Fig.33a)
- Polycarbonate impression trays (Jabbar & Co, India) (Fig.34)
- Metal balls – 4 mm diameter(Technocon Engineers Ltd, India) (Fig.36 a)
- Cyanoacrylate glue (Fewikwik, Pidilite Industries, India) (Fig. 36 b)
- 19 gauge stainless steel orthodontic wire (Konark Ever Bright Dental, India) (Fig. 37 a)
- Universal orthodontic plier (Manipal pliers and hand instruments, Bangalore, India)(Fig.37 b)
- Compact disc (CD-R, 750 MB, SONY, Supremas, Malaysia) (Fig.40)

EQUIPMENTS EMPLOYED:

- Autoclave (Veenex, India) (Fig.3)
- Incubator (Accurate Scientific Instruments, Mumbai) (Fig.26a)
- Optical microscope (Labomed Vision 2000, N.K Jain Instruments Pvt Ltd, India) (Fig.30)
- SOMATOM sensation CT system (Siemens Medical Solutions Inc, Malvern, Pennsylvania) (Fig.38)
- Mimics software (Materialise ,Leuven, Belgium) (Fig.44)
- Computer (Acer, Windows XP, China) (Fig.45)
- The SPSS software package for statistical analysis (SPSS for Windows 15.0, SPSS Software Corp., Munich, Germany)

SOMATOM sensation CT system (Siemens Medical Solutions Inc, Malvern, Pennsylvania) (Fig.38):

In this study, impressions were scanned by Computed Tomography (CT) to measure the dimensional accuracy. Computed tomography (CT), also known as Computed Axial Tomography (CAT), is a painless, sophisticated x-ray procedure. CT uses a computer and a rotating x-ray device to create detailed, cross-sectional images, or slices, of organs and body parts. Multiple images are taken during a CT scan, and a computer compiles them into complete, cross-sectional pictures ("slices") of soft tissue, bone, and blood vessels. A CT machine resembles a large, square doughnut. A flat "patient couch" is situated in the circular opening, which is about 24 to 28 inches in diameter. The couch can be moved up, down, forward, and backward to position the subject for imaging.

The CT scanner itself is a circular, rotating frame with an x-ray tube mounted on one side and a banana-shaped detector mounted on the other. A fan-shaped beam of x-rays is created as the rotating frame spins the x-ray tube and detector around the patient. For each complete rotation, one cross-sectional slice of the body is acquired. As the scanner rotates, the detector takes numerous snapshots called "profiles." Typically, about 1,000 profiles are taken in one rotation. Each profile is analyzed by computer, and the full set of profiles from each rotation is compiled to form the slice which is a two-dimensional image (2D).

Mimics software (Materialise ,Leuven, Belgium) (Fig.44):

In this study, the images obtained from the CT scan were converted to three- dimensional (3D) image using Mimics software for linear measurements of dimensional changes. Mimics software allows to process and edit 2D image data (CT, μCT, MRI, etc.) to construct 3D virtual images with the utmost accuracy, flexibility and user-friendliness. The 3D reconstructed images can be rotated and also magnified using the software, which aids in taking accurate measurements of distances, angles, diameters or densities either on CT/MRI images or directly on the images obtained from the 3D reconstruction.

METHODOLOGY:

The methodology adopted for this two-part study has been divided into the following stages as given below:

I. Methodology for comparatively evaluating the antimicrobial efficacy of three different chemical disinfectants on patient-derived PVS impressions:

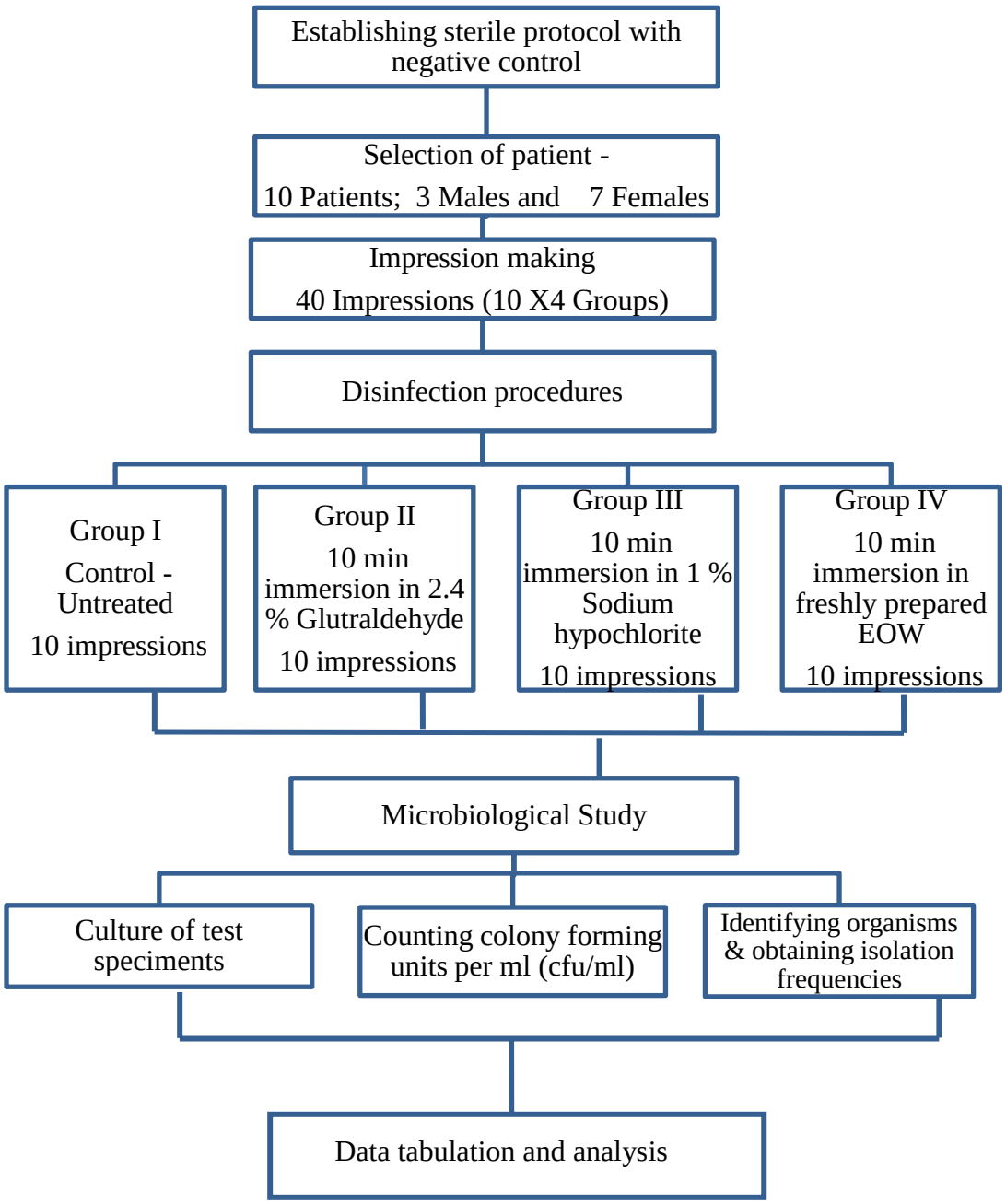
1. Establishing sterile protocol with negative control
2. Selection of Patient
3. Impression Making
4. Disinfection Procedures
5. Microbiological Study:
    a. Culture of test specimens
    b. Counting colony forming units per ml (CFU/ml)
    c. Identifying Organisms and obtaining isolation frequencies
6. Data tabulation and analysis

II. Methodology for comparatively evaluating the effect of three different chemical disinfectants on the dimensional stability of dental model-derived PVS impressions:

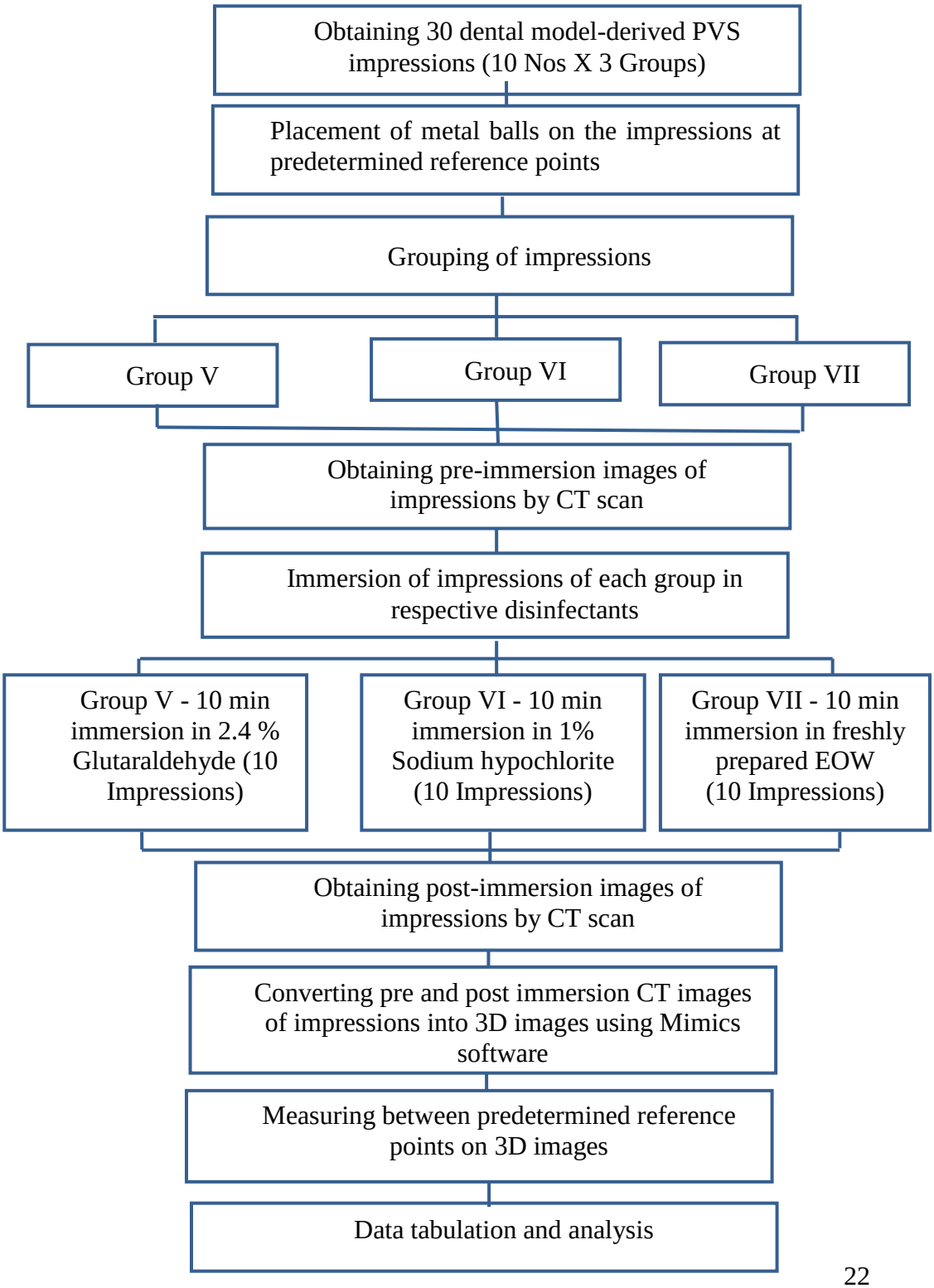
1. Obtaining 30 dental model-derived PVS impressions
2. Placement of metal balls on the impressions at predetermined reference points
3. Grouping of impressions
4. Obtaining pre-immersion images of impressions by CT scan
5. Immersion of impressions of each group in respective disinfectants
6. Obtaining post-immersion images of impressions by CT Scan
7. Converting pre and post immersion CT images of impressions into 3D images using Mimics software
8. Measuring between predetermined reference points on 3D images
9. Data tabulation and analysis

FLOW CHART

I. Methodology for comparatively evaluating the antimicrobial efficacy of three different chemical disinfectants on patient-derived PVS impressions:



II. Methodology for comparatively evaluating the effect of three different chemical disinfectants on the dimensional stability of dental model-derived PVS impressions:



I. Methodology for comparatively evaluating the antimicrobial efficacy of three different chemical disinfectants on patient-derived PVS impressions:

1. Establishing sterile protocol with negative control:

In the present study, addition-curing, polyvinyl siloxane (PVS) impression material (Affinis- Coltene Whaledent, Germany) (Fig.1a, b, c, d and e) was used as the impression material. In microbiological studies, false results can be introduced due to contamination from equipment or operator. To overcome this, the efficacy of the sterile protocol followed for obtaining impressions has to be verified. To standardize the sterile protocol used in the present study, as a negative control a PVS impression was made of the maxillary arch of a dental model with typodont teeth and rubber simulated soft tissue (Nissin Dental Products, Inc, Japan) (Fig.2), which was pre-sterilized by autoclaving (Veenex, India)(Fig.3) at 15Lbs pressure (121 deg c) for 15 minutes.

The metallic impression tray (Jabbar & Co, India) (Fig.4), nitrile gloves (Kimberly clark hygiene products Pvt. Ltd, Pune, India) (Fig.5) and polypropylene container (Parsons Pvt. Ltd Mumbai, India) (Fig. 6) employed for obtaining and storing the PVS impression were sterilized by autoclaving at 15Lbs pressure (121 deg c) for 15 minutes. The automixing gun (JSP Dental, California, USA) (Fig.1c), mixing tips (Affinis- Coltene Whaledent, Germany) (Fig.1d) and proportioning scoops (Affinis- Coltene Whaledent, Germany) (Fig 1e) required, were subjected to cold sterilization using 2.4% Glutaraldehyde (Cidex, Johnson & Johnson Ltd, India) (Fig.7). The working unit was wiped vigorously with cotton

(Ramraju Surgical Cotton Mills Ltd., Rajalaiyam, India) (Fig.8)soaked in 2.4 % glutaraldehyde before the procedure.

The impressions were made by the putty-wash technique in a single stage procedure in metallic stock impression trays. The putty and light body impression material used were proportioned and mixed according to the manufacturer's instructions. Equal volumes of base and catalyst were proportioned using scoops (Fig.1e) supplied with the material by the manufacturer and then mixed together for 45 seconds until a homogenous mix was obtained (Fig. 9a).

The tray was loaded and light body PVS was injected over the putty material (Fig.9b). Equal volumes of base and catalyst pastes were mixed together using the automixing cartridge system (Fig.1c) to provide a homogenous mix. The tray was centered and seated over the dental model and stabilized with firm finger pressure (Fig.9c). Impressions were allowed to set until permanent deformation no longer resulted from thumb nail depression (Fig.10).

The dental model-derived PVS impression was gently rinsed with 250 cc distilled water (Vijayshree traders, Chennai) (Fig.11a) taken in a calibrated beaker (Borosil Glass Works Ltd, Ahmedabad, India) (Fig. 11b) for 45 seconds and immediately transferred to an airtight sterile polypropylene container (Fig.12). The above impression was transferred to the Department of Microbiology within 30 minutes and subjected to microbiological culture (described subsequently) to detect the presence of microbial growth. No bacterial growth was detected following culture (Fig.27a). Hence the same sterile protocol was followed for

obtaining all the forty patient-derived PVS impressions required in the present study.

2. Selection of patient:

Approval for the present study was obtained from the Institutional Review Board (The Ethics Committee) of the Ragas Dental College and Hospital, Chennai, India. A total of 10 patients were selected from the Department of Prosthodontics, Ragas Dental College and Hospital, Chennai, India. Patients were selected after obtaining informed consent in accordance with ethical clinical policies. Selected group comprised of 3 males and 7 females of age ranging from 20- 50 years. (Mean age 30 years).

Patients were selected based on the following inclusion criteria:

- Medical and dental histories of the selected patients were reviewed and updated.
- Selected patients had not received any form of antibiotics or antifungal therapy for the past six months.
- Selected patients had not received any form of immunosuppressive therapy or chemotherapy.
- Selected patients were not edentulous in either jaw or wearing any prosthesis and had more than 10 teeth present in the maxillary arch. They had not received any oral hygiene measures or specific tooth brushing instructions and were not using any mouth rinse.

3. Impression making:

The same sterilization/disinfection protocol described previously was followed for obtaining all the patient-derived PVS impressions in the present study (Fig.13).

The patient chair and unit were vigorously wiped using cotton soaked in 2.4% glutaraldehyde just before each impression making procedure. Each patient was draped with sterile disposable drape (Plasti Surge Industries Pvt. Ltd, Amravati,India) (Fig.14a) and head cap (Crosscare Surgical Disposables, Chennai, India) (Fig.14b) before making each impression. The operator was draped with sterile disposable drape, head cap and face mask (Crosscare Surgical Disposables, Chennai India) (Fig.14c) and sterile nitrile gloves before making each impression.

Four PVS impressions were made of the maxillary arch for each of the 10 patients randomly on four different days separated by a duration not less than 72 hours to obtain a total of 40 impressions. The impressions were made by the putty-wash technique in a single stage procedure in sterile metallic impression trays.

Both the putty and light body impression material used were proportioned and mixed according to the manufacturer's instructions. The procedures for manipulation and tray loading were similar to that described previously for typodont model. The tray was inserted into the mouth and seated and stabilized with firm finger pressure till set. Impressions were allowed to set until permanent deformation no longer resulted from thumb nail depression (Fig.15).

4. Disinfection procedures:

The obtained impressions were randomly assigned into four groups as below:

i. Out of the 40 patient-derived PVS impressions**,** one impression from each patient was randomly subjected to rinsing with 250 cc distilled water for 45 seconds only and these were treated as the control group (Fig.16).

- Group I- Control / Untreated Group - 10 Impressions.

  Each of these impressions was transferred immediately to individual airtight sterile polypropylene containers (Fig.17).

ii. The remaining 30 patient-derived PVS impressions were randomly divided into three groups and immersed into three different chemical disinfectants as described below:

- One impression obtained from each patient was rinsed with 250 cc distilled water for 45 Seconds and immediately subjected to immersion in 2.4 % Glutaraldehyde (Fig.7) for 10 minutes in individual airtight sterile polypropylene containers (Fig.18).
  - Group II - 10 Impressions
- One impression obtained from each patient was rinsed with 250 cc distilled water for 45 Seconds and immediately subjected to immersion in 1% Sodium Hypochlorite (Alan Medical and Laboratory products, Chennai, India) (Fig.19a) for 10 minutes in individual airtight sterile polypropylene containers (Fig 19b).
  - Group III- 10 Impressions

- One impression obtained from each patient was rinsed with 250 cc distilled water for 45 Seconds and immediately subjected to immersion in freshly prepared Electrolyzed Oxidizing water (EOW) (Tianno Ti Anode Fabricators Pvt Ltd, Chennai, India) (Fig.20a) for 10 minutes in individual airtight sterile polypropylene containers (Fig.20b).
    - Group IV- 10 Impressions

  After the respective disinfection procedures, the disinfectants were discarded and only the impressions were stored in their respective containers (Fig.21).All the 40 patient- derived PVS impressions were transferred as and when obtained to the Department of Microbiology without further delay after their respective disinfection protocols and subjected to further microbiological study.

5. Microbiological study:

The culture, counting of colony forming units and identification of the organisms in the present study was done in the Department of Microbiology, Ragas Dental College and Hospital, Chennai, India. The following procedures were done:

a) Culture of test specimens:

Each impression was emulsified with 10 ml of the sterile saline (Nirlife Healthcare Nirma Limited, Gujarat, India)(Fig.22a) taken in a sterile calibrated cylinder (Borosil Glass Works Ltd, Ahmedabad, India) (Fig.22b) and shook for 5 minutes in their respective containers. 0.01 ml of this suspension was taken in a micropipette (Accumax, fine care bio systems, Gujarat, India) (Fig.23a) and

individually plated and streaked using calibrated wire loop (Himedia Laboratories Ltd, Mumbai, India)(Fig.23b,c) on Brain Heart Infusion Agar(BHI Agar)(Himedia laboratories Ltd, Mumbai, India) (Fig.24a).

The BHI agar was prepared according to the manufacturer's instructions as below:

- 52 gm of the powder was suspended in 1000 ml of distilled water.
- Then, it was heated to boiling to dissolve the medium completely.
- Sterilized by Autoclaving at 15 Lbs pressure (121 Deg C) for 15 minutes and mixed well and then poured into the plates (Fig.24 b, c and d). The plates were marked with glass marking pencil (Hindustan Pencils Ltd, Mumbai) to aid in future identification (Fig. 25a and b). In this manner, a total of 40 culture plates were prepared for each of the 40 test impressions belonging to 4 test Groups (Groups I to IV).

The marked plates were transferred to an incubator (Accurate Scientific Instruments, Mumbai) (Fig.26a) and incubated for 24 hrs at 37° C (Fig.26b). At the end of 24 hrs, plates which exhibited no growth were subjected to further incubation for 24 hrs. At the end of 48 hrs, the plates were removed from the incubator and microbial growth analyzed (Fig.27 b - i).

b) Counting colony forming units per ml (cfu/ml):

Each marked plate was individually studied for microbial growth. Number of colonies was counted by visual observation (Fig.27 b - i). Where the colonies

were too numerous to count, the method of serial dilution was employed for counting.

- In this method, the specimen was diluted in saline in the ratio 1: 10 in test tubes (Borosil Glass Works Ltd, Ahmedabad, India) (Fig.28). After dilution, the specimen was streaked on the culture plate with a calibrated wire loop and incubated for 24 hrs at 37 Deg C. Then, the colonies were observed and counted.
- The number of colonies multiplied by the "dilution factor" (the number of times that the bacteria sample has been diluted with the diluent sample) gave the number of colony forming units per ml for each specimen.

C) Identifying organisms:

Subsequent to counting of the colonies, the type of colonies was identified by visual observation (Fig. 27 b-i).

Gram staining was done (Fig. 29 a and b) and slides were observed under an optical microscope (Labomed Vision 2000, N.K Jain Instruments Pvt Ltd, India) (Fig. 30) under 40 X magnification (Fig. 31 a-f). Gram negative organisms were further identified by biochemical tests.

Where the isolate was Candida, Sabouraud agar Medium (Himedia Laboratories Ltd, Mumbai, India) (Fig.32) was used for confirmation. Tiny colonies on BHI were picked up and subcultured on blood agar plates (Fig. 33a,b,c and d) and identified to see whether they were streptococcus or

others. Smear was also done for confirmation. The isolation frequencies (in percentage) of each type of organism for each group were also noted.

6. Data tabulation and analysis:

The number and type of microbial colonies were individually tabulated for all the 40 specimens belonging to Groups I, II, III and IV and the data obtained was statistically analyzed. The microbial colony count (log_{10} values) for each sample was tabulated based on the number of colony forming units observed and the mean for each test group obtained.

The log_{10} reduction for the three chemical disinfectant groups (Group II, III and IV) were obtained and statistically compared with that for the control group (Group I) and with each other. The kill rate % was also calculated for the three disinfectant groups.

All statistical calculations were performed using Microsoft Excel 2007 (Microsoft, USA). The SPSS (SPSS for Windows 15.0, SPSS Software Corp., Munich, Germany) software package was used for statistical analysis. All statistical analysis for test of significance were performed using One-way ANOVA followed by multiple comparisons between test groups using Tukey-HSD Post-hoc tests and a P value < 0.05 was considered statistically significant.

II. Methodology for comparatively evaluating the effect of three different chemical disinfectants on the dimensional stability of dental model-derived PVS impressions:

1. Obtaining 30 dental model-derived PVS impressions:

Polyvinyl siloxane impressions were made of the maxillary arch of a dental model with typodont teeth and rubber-simulated soft tissue (Fig.2). The impressions were made by the putty-wash technique in a single stage procedure in polycarbonate impression trays (Jabbar & Co, India) (Fig.34). The procedures for mixing and loading the tray with the putty and light body consistencies were similar to that described previously.

The tray was centered and seated over the dental model and stabilized with firm finger pressure. Impressions were allowed to set until permanent deformation no longer resulted from thumb nail depression and then separated from the model (Fig.35). In this manner, a total of 30 PVS impressions were obtained.

2. Placement of metal balls on the impressions at predetermined reference points:

Four metal balls, 4 mm in diameter (Technocon Engineers Ltd, India) (Fig. 36 a) were secured with cyanoacrylate glue (Fewikwik, Pidilite Industries, India) (Fig. 36 b) on the impressions at four predetermined reference points to facilitate the measurement of distance between them (Fig. 36 c). The reference points used in the present study were the cusp tip of right and left canines and mesiobuccal cusp tip of right and left first molar.

The impressions were marked to aid in future identification. 19 gauge orthodontic wires (Konark Ever Bright Dental, India) (Fig.37 a) were bent into alphabets A-J with a universal orthodontic plier (Manipal pliers and hand instruments, Bangalore, India) (Fig. 37 b). The alphabets were secured on the handle of the respective impression trays with cyanoacrylate glue. To distinguish within the groups before and after the disinfection procedures, the impressions were marked as A-J before immersion and A1-J1 after immersion (Fig. 37 c).

3. Grouping of impressions:

The 30 impressions were randomly divided into three groups as Group V, Group VI and Group VII (10 Impressions per group). The same set of ten impressions in each group was subjected to pre and post immersion CT Scans.

4. Obtaining pre-immersion images of impressions by CT scan:

3D computed tomography scanner (Siemens SOMATOM Sensation 64 Slice) (Fig.38) was used to record the pre-immersion images of all the 30 dental model-derived PVS impressions (Groups V, VI and VII). Each group (10 impressions) was recorded in one single scan (Fig. 39).

The dental model-derived impressions were placed on the flat "patient couch" with a supportive cardboard underneath the impressions as the scanner bed was not flat. The impressions were arranged in two columns of five each ensuring adequate space between two impressions.

The images are taken in the sharpest algorithm in the CT machine (sinus algorithm, slice thickness, 0.50mm: 120kv and 225 and 250mA (anterio-

posterior-latero lateral,H70h). Each row data was stored in DICOM format in a separate compact disc (SONY, 750 MB) (Fig.40 and 41).

5. Immersion of impressions of each group in respective disinfectants:

After the impressions were scanned, they were immersed in their respective disinfectants (3 Groups X 10 impressions). The 10 impressions of Group V were immersed in 2.4 % glutaraldehyde for 10 minutes (Fig. 42a). The 10 impressions of Group VI were immersed in 1% Sodium hypochlorite for 10 minutes (Fig. 42 b). The 10 impressions of Group VII were immersed in freshly prepared Electrolyzed Oxidizing water (EOW) for 10 minutes (Fig. 42 c).

6. Obtaining post-immersion images of impressions by CT scan:

Post-immersion CT scan was obtained for all the 30 dental model derived impressions after their respective disinfection procedures. The procedure followed was similar to that employed for obtaining pre-immersion images. The post-immersion scanned images were recorded in standard DICOM format in compact disc (Fig.43).

7. Converting pre and post immersion CT images of impressions into 3D images using Mimics software:

DICOM raw data sets were reconstructed using Mimics software (Materialise, Leuven, Belgium) (Fig.44) to obtain 3D images. All the pre and post immersion CT images from the compact discs were copied to the computer (Acer, Windows XP, China) (Fig. 45). Computed tomography data were imported into Mimics soft ware.

The software reconstructed the CT scan image data into three dimensional (3D) digital images. Color images for each scanned impression were obtained.

8. Measuring between predetermined reference points on 3D images:

The Mimics software facilitates the linear measurement of distances between any chosen reference points. In the present study, this software was used for linear measurements between the following predetermined reference points on all the converted 3D images of all Groups (Groups V, VI and VII) (Fig. 46 a & b 47a & b and 48a & b).

- Inter-canine distance: Cusp tip of right canine – Cusp tip of left canine.
- Right canine – Right molar distance: Cusp tip of right canine – mesiobuccal cusp tip of right first molar.
- Left canine – Left molar distance: Cusp tip of left canine – mesiobuccal cusp tip of left first molar.
- Inter-molar distance: mesiobuccal cusp tip of right first molar – mesiobuccal cusp tip of left first Molar.

The software facilitated direct linear measurement of these distances between the centers of the metal balls placed at the above predetermined reference points. For measuring the above distances, a built-in tool in the Mimics software (measure 3D distance tool) was selected and by clicking and dragging on the selected points, the measurement between the points was automatically obtained. All measurements for each image were saved for future reference. All the measurements were noted down for data analysis.

9. Data tabulation and analysis:

The data obtained from the above linear measurements for all the samples of each test group were tabulated and the mean for each measurement obtained and subjected to statistical analysis. All statistical calculations were performed using Microsoft Excel 2007 (Microsoft, USA). The SPSS (SPSS for Windows 15.0, SPSS Software Corp., Munich, Germany) software package was used for statistical analysis. All statistical analysis were performed using One-way ANOVA followed by paired sample t-test and Tukey-HSD Post-hoc tests for comparing pre and post immersion dimensional changes within and between groups and a p- value < 0.05 was considered statistically significant.

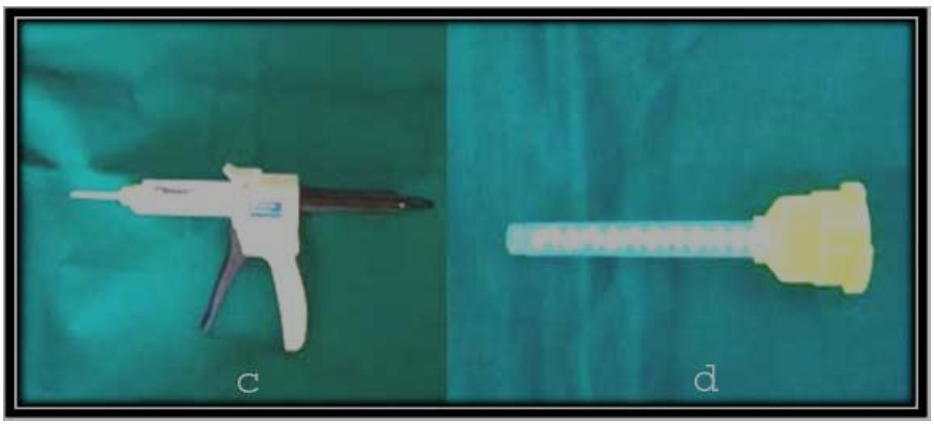


Fig.1a: Putty super soft consistency

Fig.1b: Light body consistency

Fig.1c: Auto mixing gun

Fig.1d: Mixing tip

Fig.1e: Proportioning scoops

Fig.2: Maxillary model with typodont teeth

Fig.3: Autoclave

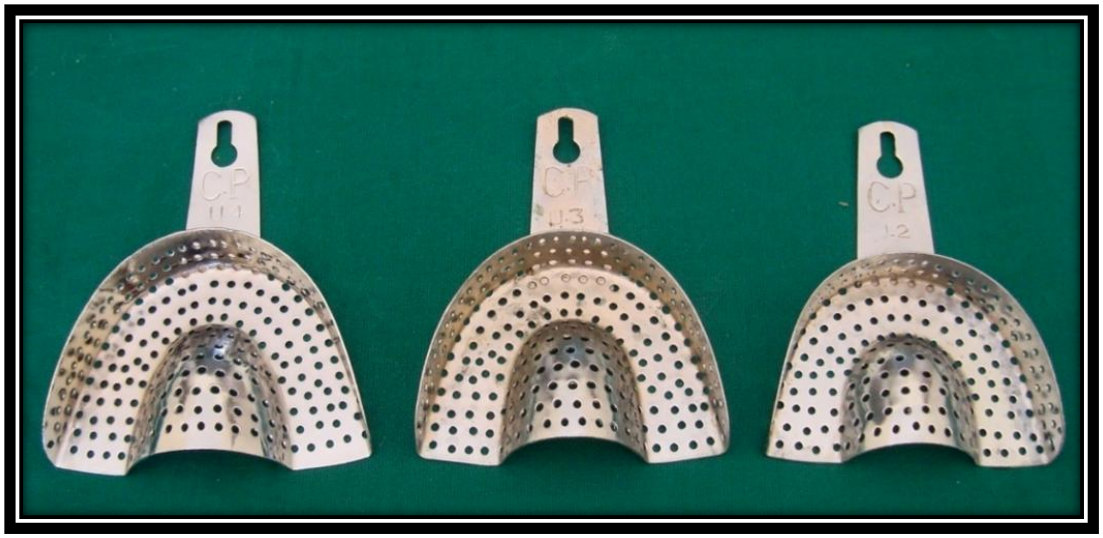


Fig.4: Metallic impression trays

Fig.5: Nitrile gloves

Fig.6: Polypropylene container

Fig.7: 2.4 % Glutaraldehyde solution

Fig.8: Cotton roll

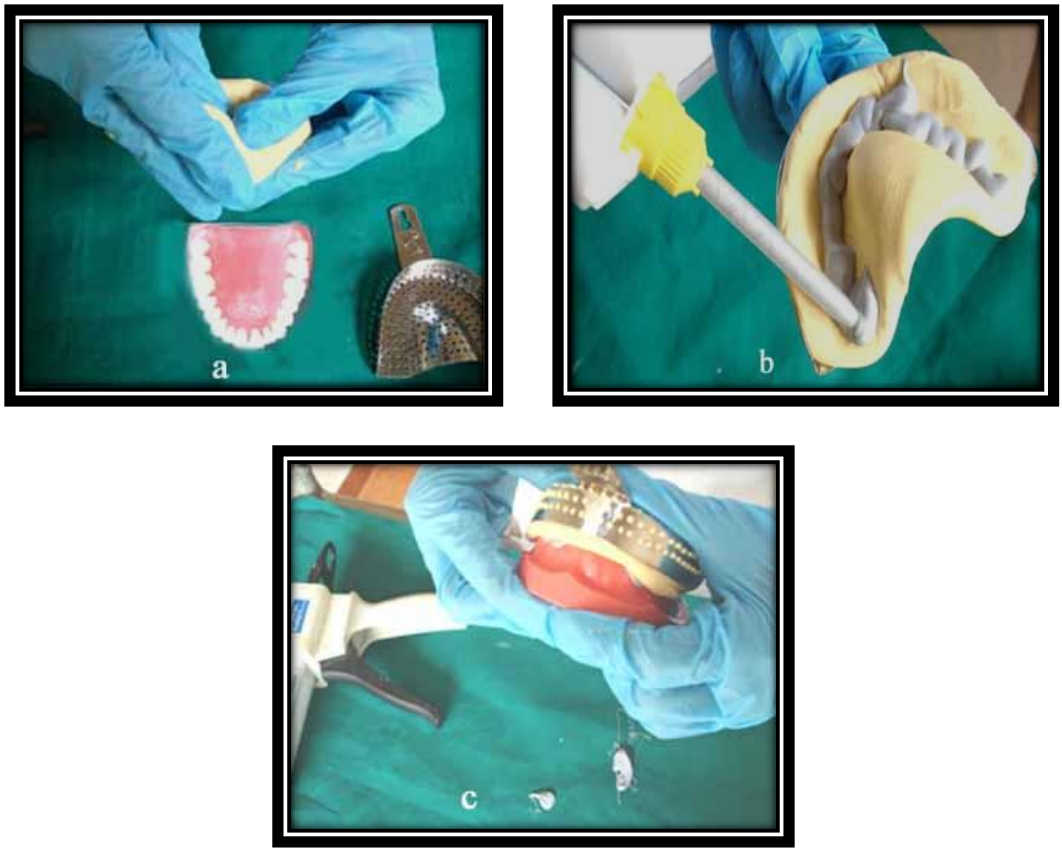


Fig.9a: Manipulation of putty

Fig.9b: Light body injected over putty loaded in impression tray

Fig.9c: Stabilizing impression tray over typodont model

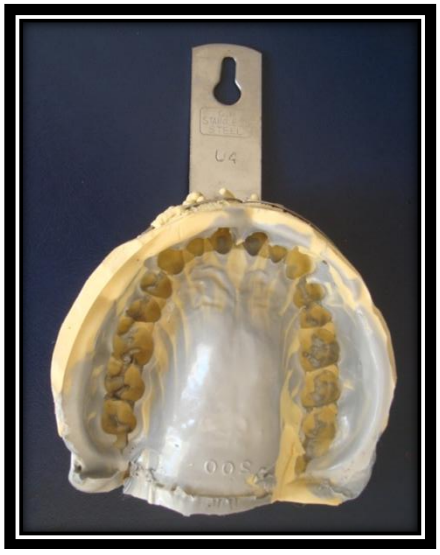


Fig.10: Dental model-derived PVS impression for negative control

Fig.11a: Distilled water

Fig.11b: Calibrated beaker

Fig.12: Impression transferred to Polypropylene container

Fig.13: Setup prior to impression making

Fig.14a: Disposable drape

Fig.14b: Disposable head cap

Fig.14c: Disposable face mask

Fig.15: Patient-derived PVS impression

Fig.16: Rinsing of PVS impression with distilled water

Fig.17: Impression transferred to polypropylene container

Fig.18: Impression immersed in 2.4 % glutaraldehyde

Fig.19a: 1% Sodium hypochlorite solution

Fig.19b: Impression immersed in 1% sodium hypochlorite

Fig. 20a: Freshly prepared electrolyzed oxidizing water (EOW)

Fig. 20b: Impression immersed in freshly prepared EOW

Fig. 21: Impression stored in polypropylene container after disinfection

Fig.22a: Saline

Fig. 22b: Calibrated cylinder

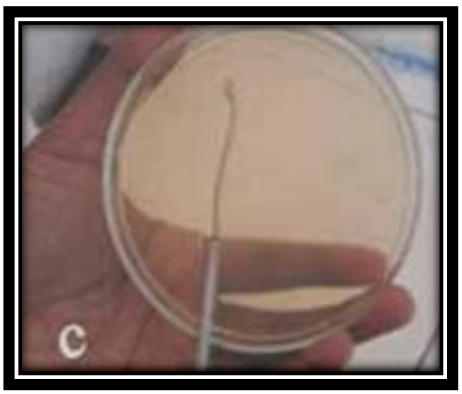


Fig.23a: Micropipette

Fig.23b: Calibrated wire loop

Fig.23c: Streaking on plate with calibrated wire loop

Fig 24a: Brain heart infusion agar (BHI)

Fig.24b: Culture plate

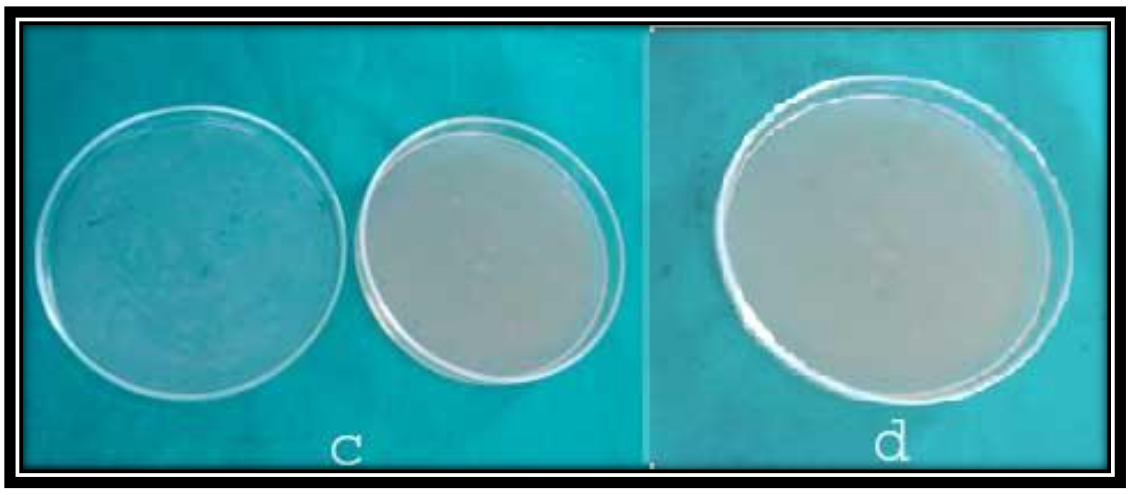


Fig. 24c: Prepared culture plate-open

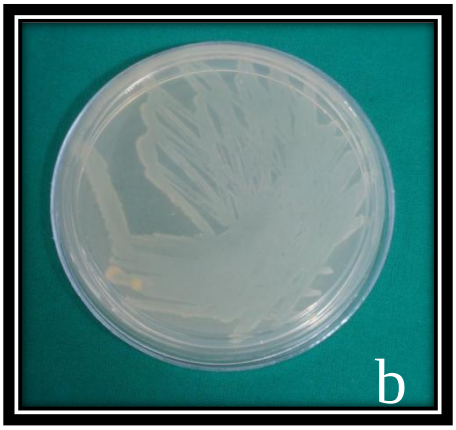
Fig. 24d: Prepared culture plate-closed

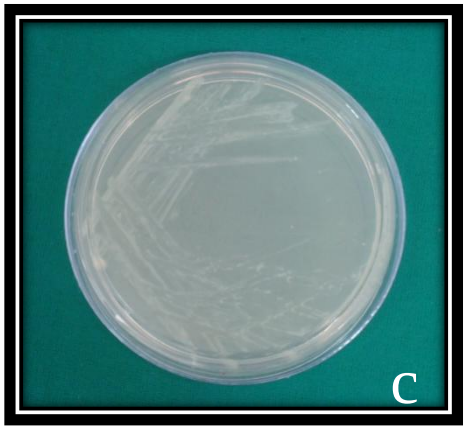
Fig.25a: Glass marking pencil

Fig.25b: Marked culture plate

Fig.26a: Incubator

Fig.26b: Culture plates placed in incubator





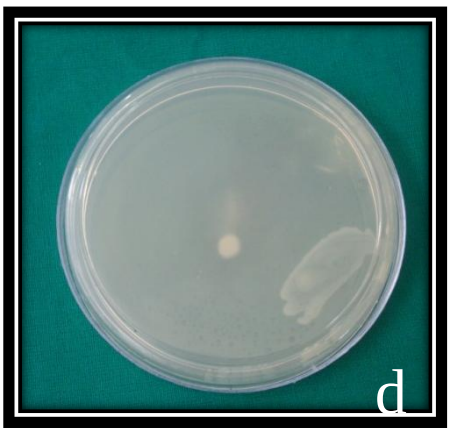


Fig.27a: Culture plate with no growth

Fig.27b: Staphylococcus aureus

Fig.27c: Candida albicans

Fig.27d: E-coli

Fig.27e:Pseudomonas aeruginosa (green pigment) and E-coli

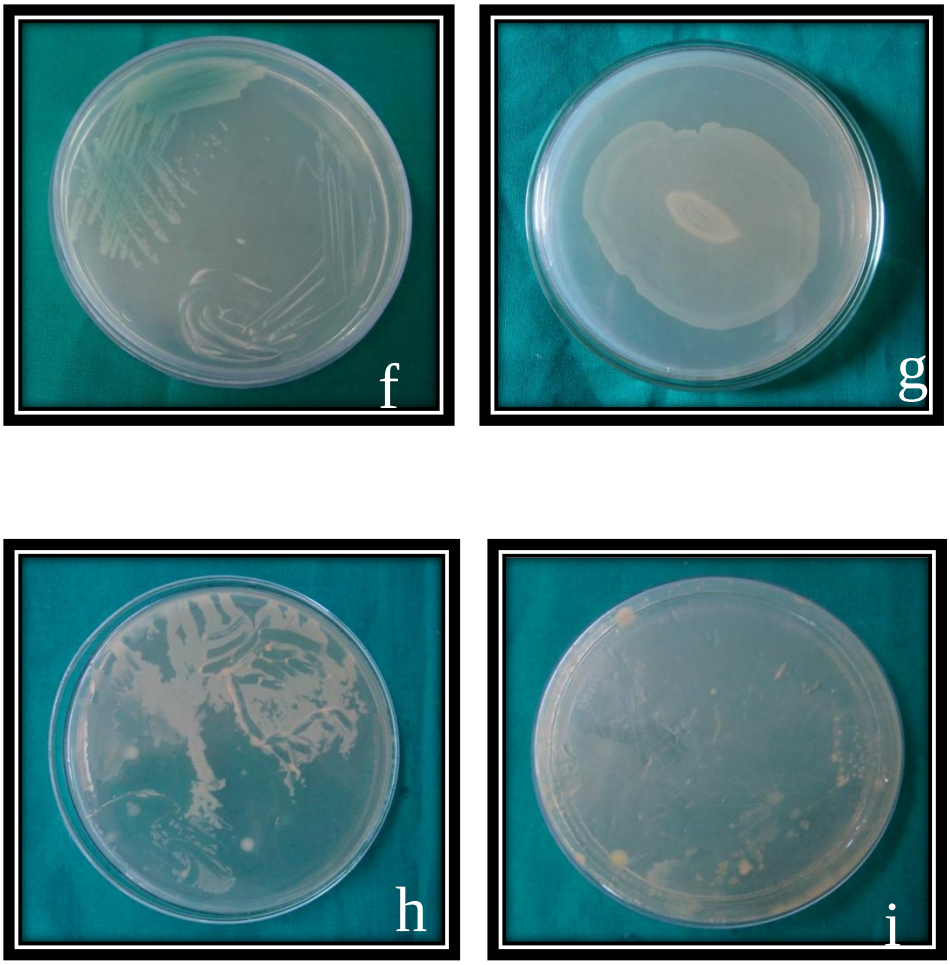


Fig.27f: Pseudomonas aeruginosa (green pigment)

Fig.27g: Proteus

Fig.27h: Staphylococcus aureus, Klebsiella, Candida albicans and Pseudomonas aeruginosa (green pigment)

Fig.27i: E-coli & Candida albicans

Fig.28: Test tubes in test tube stand

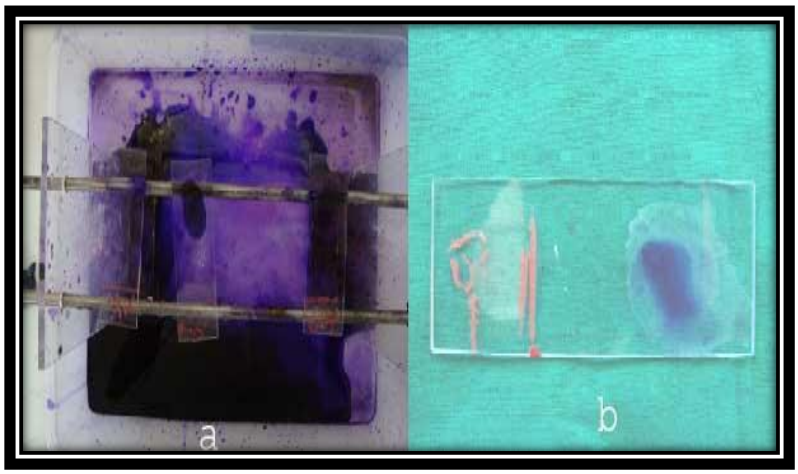


Fig.29a: Gram staining procedure

Fig.29b: Slide with completed gram staining

Fig.30: Optical microscope

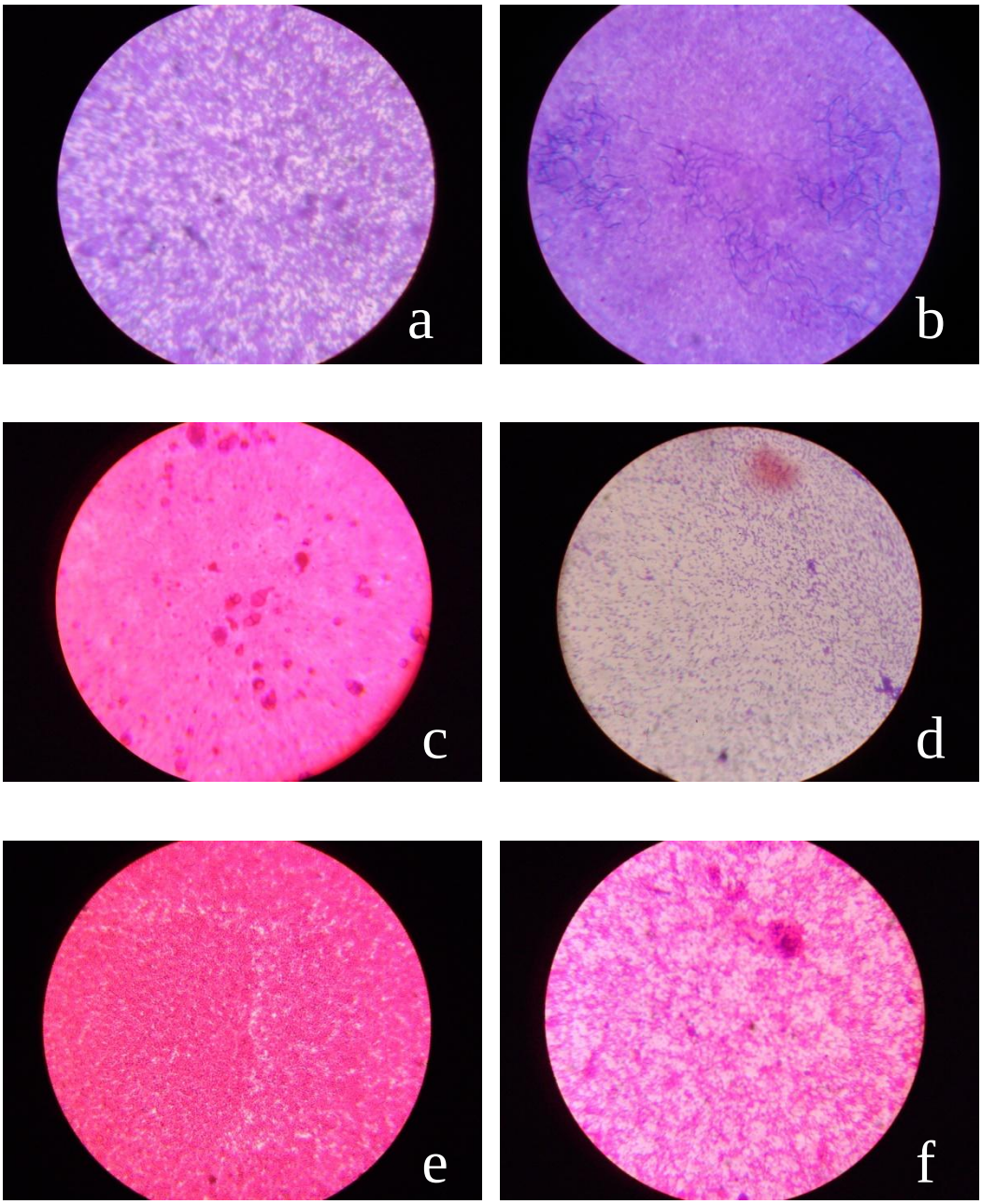


Fig.31a: Staphylococcucs aureus

Fig.31b: Candida albicans

Fig.31c: Proteus

Fig.31d: Streptococcus

Fig.31e: E-Coli

Fig.31f: Pseudomonas aeruginosa

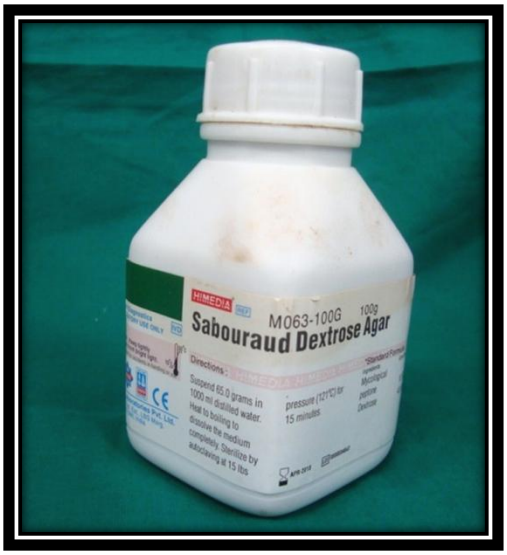


Fig.32: Sabouraud agar

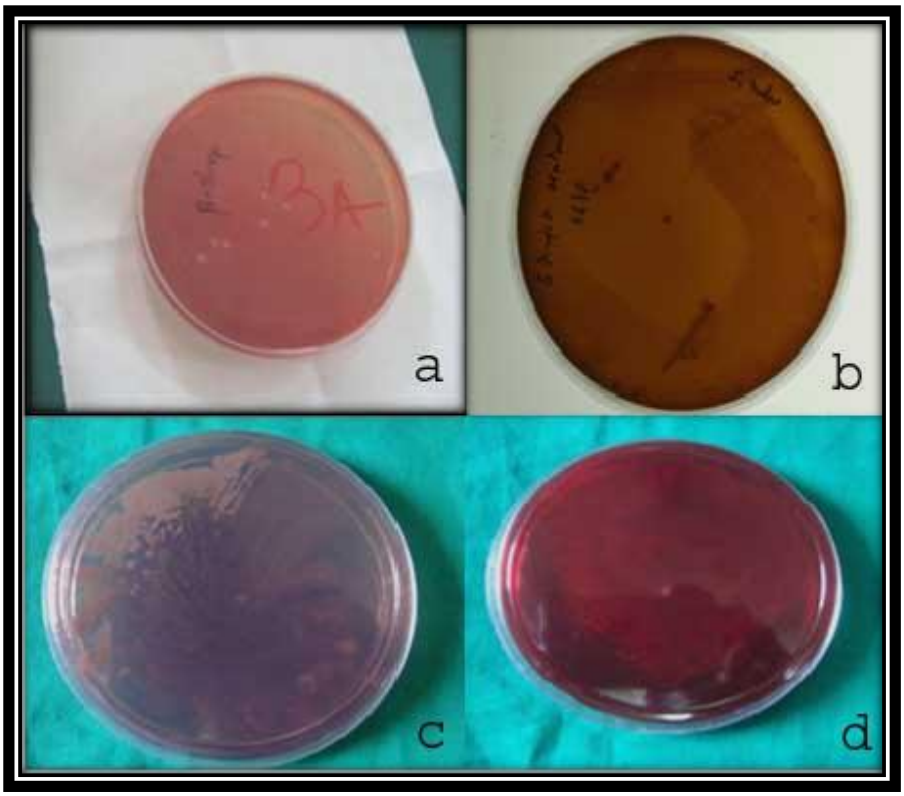


Fig.33a: Blood agar

Fig.33b: Streptococcus

Fig.33c: Pseudomonas aeruginosa (green pigment)

Fig.33d: Proteus

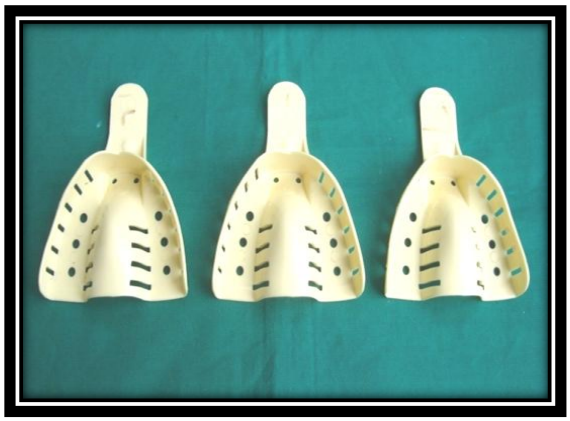


Fig.34: Polycarbonate impression trays

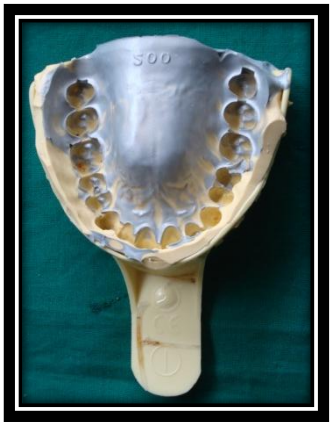


Fig.35: Dental model-derived PVS impression

Fig.36a: Metal balls

Fig.36b: Cyanoacrylate glue

Fig.36c: Four metal balls placed on the impression at reference points

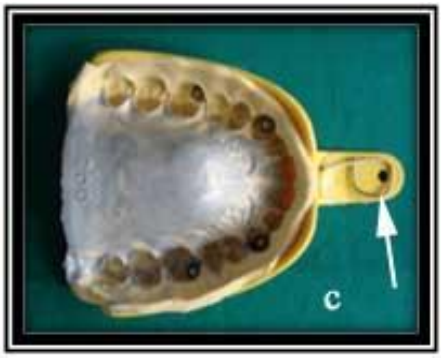


Fig.37a: 19 gauge stainless steel orthodontic wire

Fig.37b: Universal orthodontic plier

Fig.37c: Dental model-derived PVS impression marked with alphabets to aid in identification

Fig. 38: SOMATOM sensation CT system

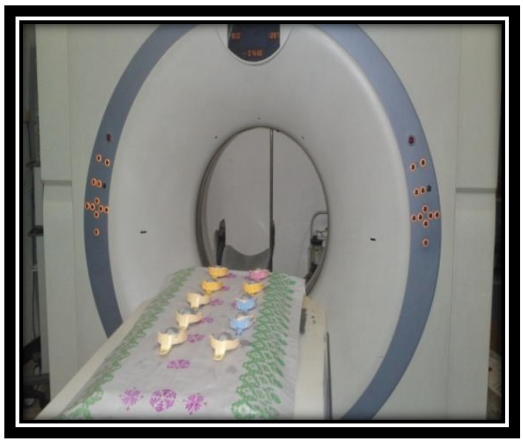


Fig. 39: CT scanning of impressions

Fig.40: Compact disc

Fig.41: Scanned images of impressions in dicom format (Pre-immersion)

Fig.42a: Immersion of dental model-derived PVS impressions in 2.4% glutaraldehyde

Fig.42b: Immersion of dental model-derived PVS impressions in 1% sodium hypochlorite

Fig.42c: Immersion of dental model-derived PVS impressions in freshly prepared EOW

Fig.43: Scanned images of impressions in dicom format (Post- immersion)

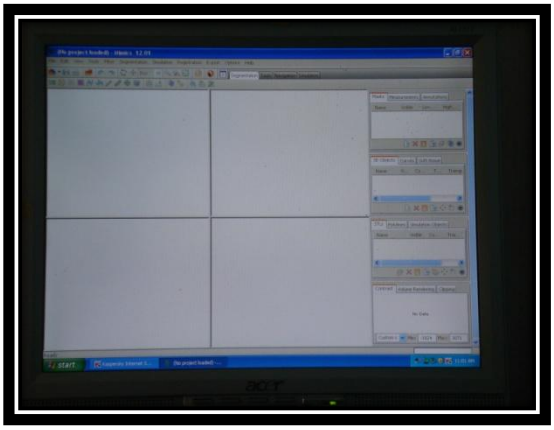


Fig.44: Mimics software

Fig.45: Computer

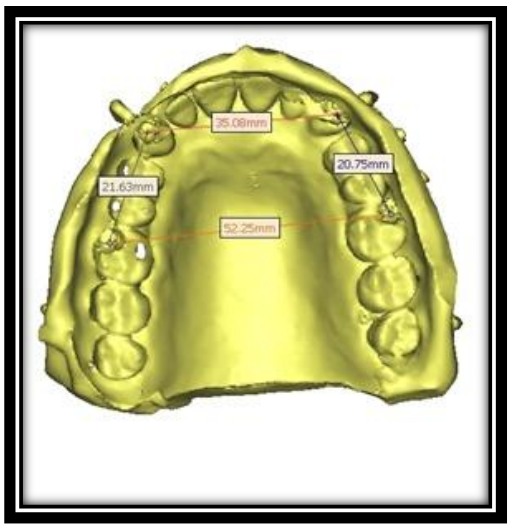


Fig.46a: 3D image of pre-immersion impression of Group V showing measurements

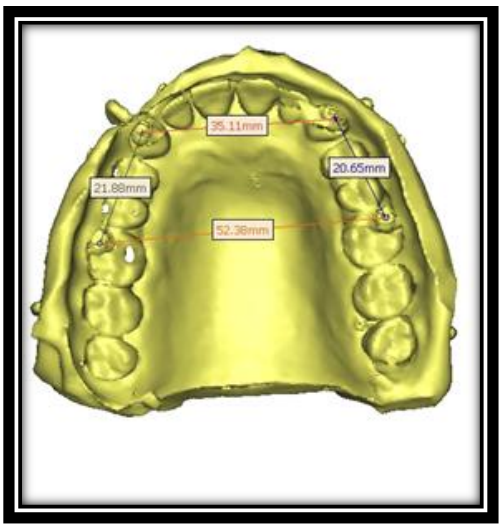


Fig.46b: 3D image of post- immersion impression of Group V showing measurements

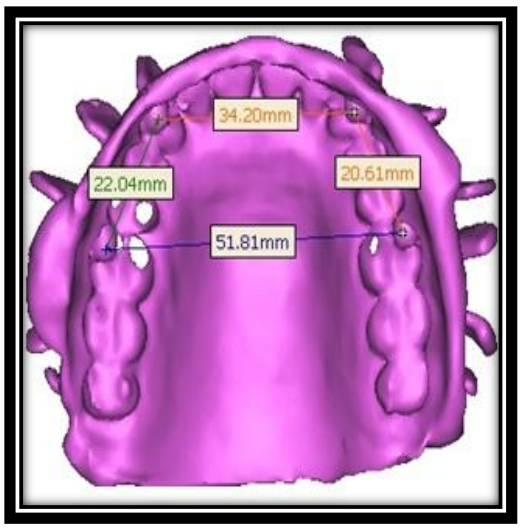


Fig.47a: 3D image of pre- immersion impression of Group VI showing measurements

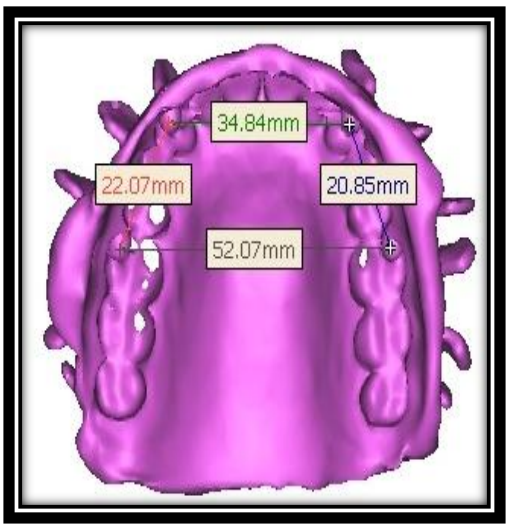


Fig.47b:3D image of post-immersion impression of Group VI showing measurements

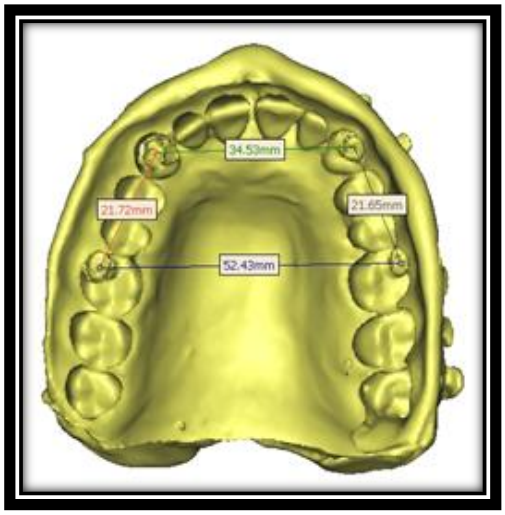


Fig 48a: 3D image of pre-immersion impression of Group VII showing measurements

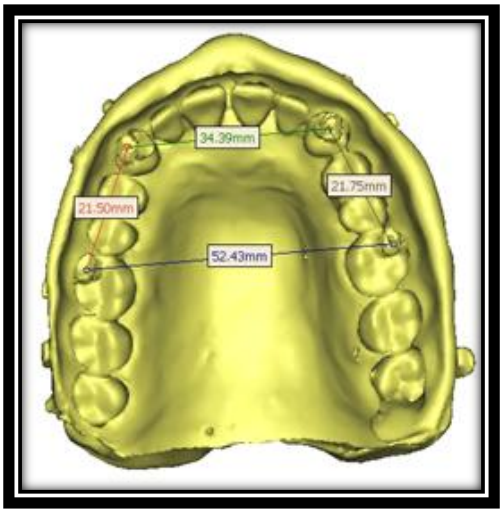


Fig.48b:3D image of post- immersion impression of Group VII showing measurements

RESULTS

The present study was conducted to comparatively evaluate the antimicrobial efficacy of three different chemical disinfectants and their effect on the dimensional stability of polyvinyl siloxane (PVS) impressions.

The results of this two-part study are tabulated under the following sections:

I. Antimicrobial efficacy of three different chemical disinfectants on patient-derived PVS impressions.

II. Effect of three different chemical disinfectants on the dimensional stability of dental model-derived PVS impressions.

I. Results of the antimicrobial efficacy of three different chemical disinfectants on patient-derived PVS impressions:

- A total of 40 patient-derived PVS impressions (4 impressions from each patient) were obtained and divided into four test groups. Each test group had one impression from each patient.
- The test groups were:

  Group I - Control or untreated impressions (10 samples)

  Group II - Impressions immersed for 10 minutes in 2.4 % Glutaraldehyde (10 samples)

  Group III - Impressions immersed for 10 minutes in 1% Sodium hypochlorite (10 samples)

  Group IV- Impressions immersed for 10 minutes in freshly prepared EOW (10 Samples).

The above test groups were subjected to microbiological study and the results were quantitatively and qualitatively obtained as follows:

1. Quantitative results
    a. Obtaining colony forming units per ml (cfu/ml).
    b. Converting colony forming units per ml (cfu/ml) into log $_{10}$ count values.
    c. Obtaining reduction in log $_{10}$ count values.
    d. Obtaining kill rate percentage.
2. Qualitative results
    a. Identification of type of microorganisms.
    b. Isolation frequencies (in percentage) of micro organisms within Groups.

Table 1 shows Basic data of colony forming units per ml (cfu/ml) for Groups I, II, III and IV.

Table 2 shows Mean, Standard Deviation and p-value of colony forming units per ml (cfu/ml) obtained for Groups I, II, III and IV.

Table 3 shows Mean log count values (log $_{10}$), Standard Deviation and p-value for Groups I, II, III and IV.

Table 4 shows Mean log count (log $_{10}$) reduction values, Standard Deviation and p-value for Groups II, III and IV.

Table 5 shows multiple comparisons of log count (log $_{10}$) reduction values obtained for Groups II, III and IV.

Table 6 shows kill rate % for Groups II, III and IV.

Table I: Basic data of colony forming units per ml (cfu/ml) for Groups I, II, III and IV

S No	Microbial count			
	Group I	**Group II**	**Group III**	**Group IV**
1	10000000	100000	10000	0
2	10000000	2000	2000	0
3	10000000	100000	100000	0
4	10000000	0	0	0
5	30000	0	1000	0
6	10000000	0	2000	0
7	10000000	100000	31000	0
8	100000	20000	0	0
9	10000000	2000	0	0
10	10000000	0	0	0

Table 2: Mean, Standard Deviation and p-value of colony forming units per ml (cfu/ml) obtained for Groups I, II, III and IV

	N	**Mean**	**Std. Deviation**	**P Value**
Group I	10	8013000.0000	4188996.29983	< 0.001 **
Group II	10	32400.0000	47034.73893	
Group III	10	14600.0000	31514.37062	
Group IV	10	0.0000	0.00000	

Note: ** denotes significance at 1 % level (highly significant)

Table 3: Mean log count ($\log_{10}$) values, Standard Deviation and p-value for Groups I, II, III and IV

Groups	N	Mean	Std. Deviation	p-value
Group I	10	6.5477	0.96144	0.001**
Group II	10	2.5903	2.31410	
Group III	10	2.3093	2.07191	
Group IV	10	0.0000	0.00000	

Note: ** denotes significance at 1 % level (highly significant)

Inference:

The difference in mean colony forming units and log count ($\log_{10}$) values obtained for Groups I, II, III and IV were significantly different from each other.

Table 4: Mean log count ($\log_{10}$) reduction values, Standard Deviation and p-value for Groups II, III and IV

	N	Mean	Std. Deviation	p-value
Group II	10	3.9574	2.36379	0.010**
Group III	10	4.2384	2.13963	
Group IV	10	6.5477	0.96144	

Note: ** denotes significance at 1 % level (highly significant)

Table 5: Multiple comparisons of log count (log $_{10}$) reduction values obtained for Groups II, III and IV

Groups	Groups	Mean Difference	Std. Error	p-value
Group II	Group III	-0.2810	0.85984	0.943
	Group IV	-2.5903	0.85984	0.015*
Group III	Group II	0.2810	0.85984	0.943
	Group IV	-2.3093	0.85984	0.032*
Group IV	Group II	2.5903	0.85984	0.015*
	Group III	2.3093	0.85984	0.032*

Note: * denotes significance at 5% level; p-value < 0.05

Inference:

The mean log count (log $_{10}$) reduction values obtained for Groups II and III were statistically insignificant. The mean log count (log $_{10}$) reduction values obtained for Group IV were statistically significant when compared to Groups II and III.

Table 6: Kill rate % for Groups II, III and IV

Group	Kill rate %
Group II	99.60 %
Group III	99.82 %
Group IV	100.00 %

II. Results of the effect of three different chemical disinfectants on the dimensional stability of dental model- derived PVS impressions:

- A total of 30 dental model derived-PVS impressions (10 impressions X 3 Groups) were obtained and randomly divided into three test groups (Group V, VI and VII).
- The impressions were subjected to immersion in disinfectants as below:
    - Group V specimens subjected to immersion in 2.4 % glutaraldehyde
    - Group VI specimen subjected to immersion in 1 % sodium hypochlorite
    - Group VII specimen subjected to immersion in freshly prepared EOW
- The impressions of each test group were subjected to pre and post immersion CT scanning and 3D image reconstruction using Mimics software to study dimensional stability.
- The following linear measurements were done on each sample image
    - Inter-canine distance: cusp tip of right canine – cusp tip of left canine.
    - Right canine – Right molar distance: cusp tip of right canine – mesiobuccal cusp tip of right first molar.
    - Left canine – Left molar distance: cusp tip of left canine – mesiobuccal cusp tip of left first molar.
    - Inter-molar distance: mesiobuccal cusp tip of right first molar – mesiobuccal cusp tip of left first molar.
- The results were tabulated for all the test groups and analyzed.

Table 7 shows Basic data of pre and post immersion linear measurements between predetermined reference points for Group V.

Table 8 shows Mean, Standard Deviation and p-value of pre and post immersion linear measurements between predetermined reference points for Group V.

Table 9 shows Basic data of pre and post immersion linear measurements between predetermined reference points for Group VI.

Table 10 shows Mean, Standard Deviation and p-value of pre and post immersion linear measurements between predetermined reference points for Group VI.

Table 11 shows Basic data of pre and post immersion linear measurements between predetermined reference points for Group VII.

Table 12 shows Mean, Standard Deviation and p-value of pre and post immersion linear measurements between predetermined reference points for Group VII.

Table 13 shows Mean difference, Standard Deviation and p-value of pre and post immersion linear measurements between predetermined reference points for Groups V, VI and VII.

Table 14 shows Multiple Comparisons of the mean differences of pre and post immersion linear measurements between predetermined reference points for Groups V, VI and VII.

Table 7: Basic data of pre and post immersion linear measurements between predetermined reference points for Group V

S No	Canine- Canine			Canine(R)–Molar(R)			Canine (L)- Molar (L)			Molar - Molar		
	Before	After	Diff	Before	After	Diff	Before	After	Diff	Before	After	Diff
A	34.48	34.48	0.00	20.72	20.79	0.07	21.22	21.06	-0.16	52.12	52.24	0.12
B	34.81	34.81	0.00	21.4	21.56	0.16	20.79	21.07	0.28	51.64	51.78	0.14
C	34.88	35.07	0.19	21.48	21.42	-0.06	20.22	20.34	0.12	52.29	51.95	-0.34
D	34.41	34.64	0.23	21.55	21.55	0.00	21.25	21.38	0.13	52.25	52.25	0.00
E	35.27	34.96	-0.31	21.25	21.28	0.03	20.82	20.72	-0.10	52.47	52.15	-0.32
F	35.22	35.3	0.08	21.94	21.94	0.00	21.09	21.03	-0.06	52.14	52.16	0.02
G	35.36	35.28	-0.08	20.99	20.81	-0.18	22.17	22.17	0.00	53.26	53.19	-0.07
H	35.7	35.88	0.18	21.64	21.63	-0.01	20.65	20.65	0.00	51.93	51.83	-0.10
I	35.11	35.08	-0.03	21.88	21.63	-0.25	20.65	20.75	0.10	52.38	52.25	-0.13
J	34.35	34.45	0.10	21.9	22.09	0.19	21.02	21.03	0.01	52.6	52.57	-0.03

Table 8: Mean, Standard Deviation and p-value of pre and post immersion linear measurements between predetermined reference points for Group V

Reference points	N	Before		After		p-value
		Mean	SD	Mean	SD	
Canine-Canine	10	34.96	0.45	34.99	0.43	0.493
Canine (R)- Molar (R)	10	21.48	0.40	21.47	0.42	0.910
Canine(L)-Molar (L)	10	20.99	0.52	21.02	0.50	0.451
Molar- Molar	10	52.31	0.43	52.24	0.41	0.198

(Note: $p > 0.05$ denotes insignificance at 5 % level)

Table 9: Basic data of pre and post immersion linear measurements between predetermined reference points for Group VI

S No	Canine- Canine			Canine (R)-Molar (R)			Canine (L)-Molar (L)			Molar - Molar		
	Before	After	Diff	Before	After	Diff	Before	After	Diff	Before	After	Diff
A	34.61	34.82	0.21	22.19	22.25	0.06	21.03	21.01	-0.02	52.33	52.41	0.08
B	34.96	34.83	-0.13	20.73	21.06	0.33	21.85	21.8	-0.05	53.23	53.29	0.06
C	34.45	34.76	0.31	21.85	21.91	0.06	21.95	22.17	0.22	53.32	53.25	-0.07
D	35.42	35.62	0.20	22.97	22.84	-0.13	22.12	22.29	0.17	52.69	52.79	0.10
E	36.18	36.07	-0.11	21.57	21.63	0.06	22.00	21.96	-0.04	53.36	53.16	-0.20
F	35.53	35.39	-0.14	22.91	22.68	-0.23	20.85	20.81	-0.04	53.31	53.29	-0.02
G	35.28	35.13	-0.15	21.75	21.72	-0.03	22.29	22.11	-0.18	52.89	52.52	-0.37
H	34.99	34.74	-0.25	22.52	22.51	-0.01	21.86	22	0.14	53.48	53.45	-0.03
I	35.32	35.39	0.07	21.71	21.78	0.07	21.8	21.74	-0.06	53.28	53.46	0.18
J	34.97	35.15	0.18	21.77	21.7	-0.07	21.64	21.47	-0.17	52.83	52.9	0.07

Table 10: Mean, Standard Deviation and p-value of pre and post immersion linear measurements between predetermined reference points for Group VI

Reference points	N	Before		After		p-value
		Mean	SD	Mean	SD	
Canine-Canine	10	35.17	0.49	35.19	0.43	0.767
Canine (R)- Molar (R)	10	22.00	0.67	22.01	0.55	0.820
Canine (L)- Molar (L)	10	21.74	0.46	21.74	0.50	0.946
Molar- Molar	10	53.07	0.37	53.05	0.38	0.705

(Note: $p > 0.05$ denotes insignificance at 5 % level)

Table 11: Basic data of pre and post immersion linear measurements between predetermined reference points for Group VII

S No	Canine- Canine			Canine(R)-Molar(R)			Canine(L)-Molar(L)			Molar – Molar		
	Before	After	Diff	Before	After	Diff	Before	After	Diff	Before	After	Diff
A	34.44	34.97	0.53	21.63	21.68	0.05	21.38	21.57	0.19	52.22	52.71	0.49
B	34.37	34.63	0.26	21.16	21.4	0.24	20.96	21.22	0.26	52.39	52.35	-0.04
C	35.09	35.22	0.13	21.54	21.56	0.02	21.5	21.42	-0.08	52.38	52.28	-0.1
D	34.77	35.24	0.47	21.85	21.75	-0.10	21.03	21.04	0.01	52.61	52.72	0.11
E	35.26	3.00	-0.26	21.83	21.89	0.06	20.63	20.43	-0.2	52.71	52.58	-0.13
F	34.53	34.39	-0.14	21.72	21.5	-0.22	21.65	21.75	0.10	52.43	52.43	0
G	34.35	34.31	-0.04	21.53	21.38	-0.15	20.66	20.79	0.13	52.86	52.44	-0.42
H	34.39	34.37	-0.02	21.42	21.39	-0.03	21.5	21.59	0.09	52.53	52.36	-0.17
I	35.05	35.35	0.30	21.05	21.16	0.11	20.95	21.05	0.1	52.6	52.72	0.12
J	34.94	35.06	0.12	21.69	21.52	-0.17	20.67	20.93	0.26	52.99	52.69	-0.3

Table 12: Mean, Standard Deviation and p-value of pre and post immersion linear measurements between predetermined reference points for Group VII

Reference points	N	Before		After		p-value
		Mean	SD	Mean	SD	
Canine-Canine	10	34.72	0.34	34.85	0.39	0.132
Canine (R)- Molar (R)	10	21.54	0.27	21.52	0.21	0.684
Canine (L)- Molar (L)	10	21.09	0.39	21.18	0.41	0.093
Molar- Molar	10	52.57	0.23	52.53	0.17	0.594

(Note: $p > 0.05$ denotes insignificance at 5 % level)

Inference:

The mean of pre and post immersion linear measurements between above predetermined reference points, within Groups V, VI and VII were found to be statistically insignificant.

Table 13: Mean difference, Standard Deviation and p-value of pre and post immersion linear measurements between predetermined reference points for Groups V, VI and VII

Reference Points	Groups	N	Mean Difference	Std. Deviation	p-Value
Canine - Canine - Difference	Group V	10	0.0360	0.15925	0.417
	Group VI	10	0.0190	0.19649	
	Group VII	10	0.1350	0.25769	
Canine (R) - Molar (R) - Difference	Group V	10	-0.0050	0.13575	0.895
	Group VI	10	0.0110	0.14873	
	Group VII	10	-0.0190	0.14271	
Canine (L) - Molar (L) - Difference	Group V	10	0.0320	0.12856	0.356
	Group VI	10	-0.0030	0.13655	
	Group VII	10	0.0860	0.14485	
Molar - Molar - Difference	Group V	10	-0.0710	0.16148	0.846
	Group VI	10	-0.0200	0.16207	
	Group VII	10	-0.0440	0 .25189	

(Note: $p > 0.05$ denotes insignificance at 5 % level)

Inference:

The mean difference between pre and post immersion linear measurements between predetermined reference points for Groups V, VI and VII were found to be statistically insignificant.

Table 14: Multiple comparisons of the mean differences of pre and post immersion linear measurements between predetermined reference points for Groups V, VI and VII

Dependent Variable	Group	Group	Mean Difference	Std. Error	Sig.
Canine - Canine Difference	Group V	Group VI	0.0170	0.09323	0.982
		Group VII	-0.0990	0.09323	0.545
	Group VI	Group V	-0.0170	0.09323	0.982
		Group VII	-0.1160	0.09323	0.438
	Group VII	Group V	0.0990	0.09323	0.545
		Group VI	0.1160	0.09323	0.438
Canine (R) - Molar(R) Difference	Group V	Group VI	-0.0160	0.06373	0.966
		Group VII	0.0140	0.06373	0.974
	Group VII	Group V	0.0160	0.06373	0.966
		Group VII	0.0300	0.06373	0.886
	Group VII	Group V	-0.0140	0.06373	0.974
		Group VI	-0.0300	0.06373	0.886
Canine (L) - Molar (L) Difference	Group V	Group VI	0.0350	0.06119	0.836
		Group VII	-0.0540	0.06119	0.656
	Group VI	Group V	-0.0350	0.06119	0.836
		Group VII	-0.0890	0.06119	0.328
	Group VII	Group V	0.0540	0.06119	0.656
		Group VI	0.0890	0.06119	0.328
Molar - Molar Difference	Group V	Group VI	-0.0510	0.08786	0.832
		Group VII	-0.0270	0.08786	0.949
	Group VI	Group V	0.0510	0.08786	0.832
		Group VII	0.0240	0.08786	0.960
	Group VII	Group V	0.0270	0.08786	0.949
		Group VI	-0.0240	0.08786	0.960

(Note: $p > 0.05$ denotes insignificance at 5 % level)

Inference:

The mean difference of pre and post immersion linear measurements between Canine-Canine was found to be statistically insignificant for Groups V, VI and VII.

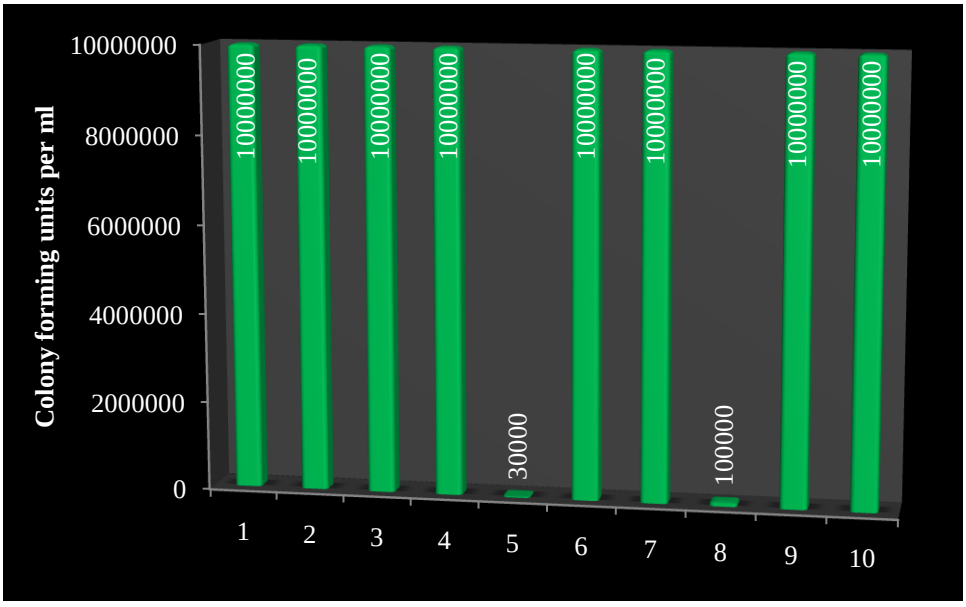
The mean difference of pre and post immersion linear measurements between Canine (R) - Molar (R) was found to be statistically insignificant for Groups V, VI and VII.

The mean difference of pre and post immersion linear measurements between Canine (L) -Molar (L) was found to be statistically insignificant for Groups V, VI and VII.

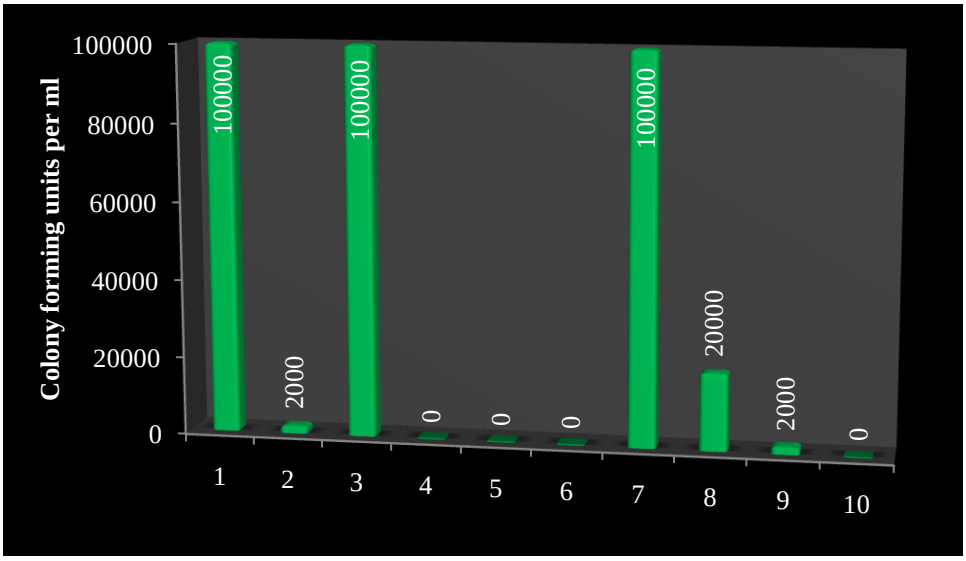
The mean difference of pre and post immersion linear measurements between Molar - Molar was found to be statistically insignificant for Groups V, VI and VII.

Graph 1: Basic data of colony forming units per ml (cfu/ml) for Groups I, II, III and IV

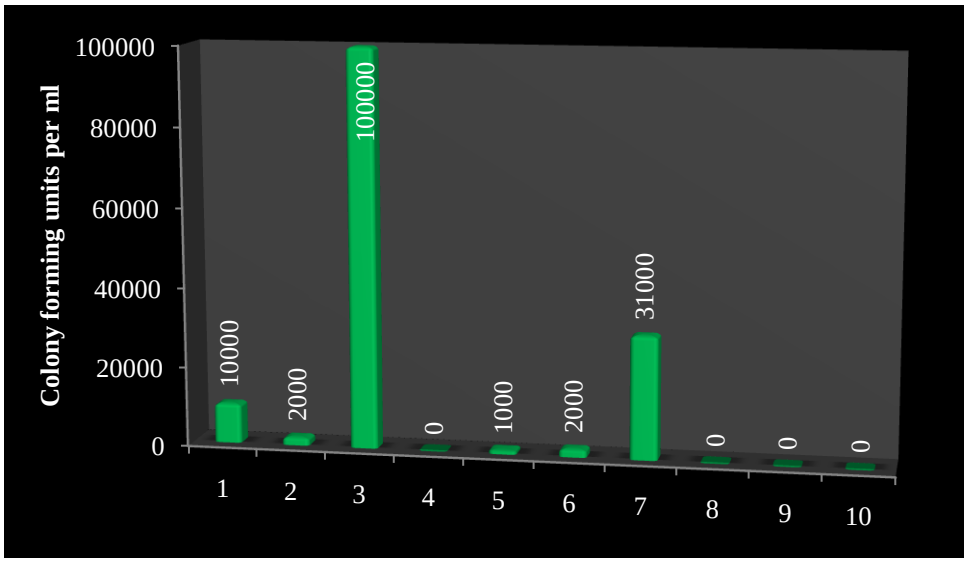
1a. Group I



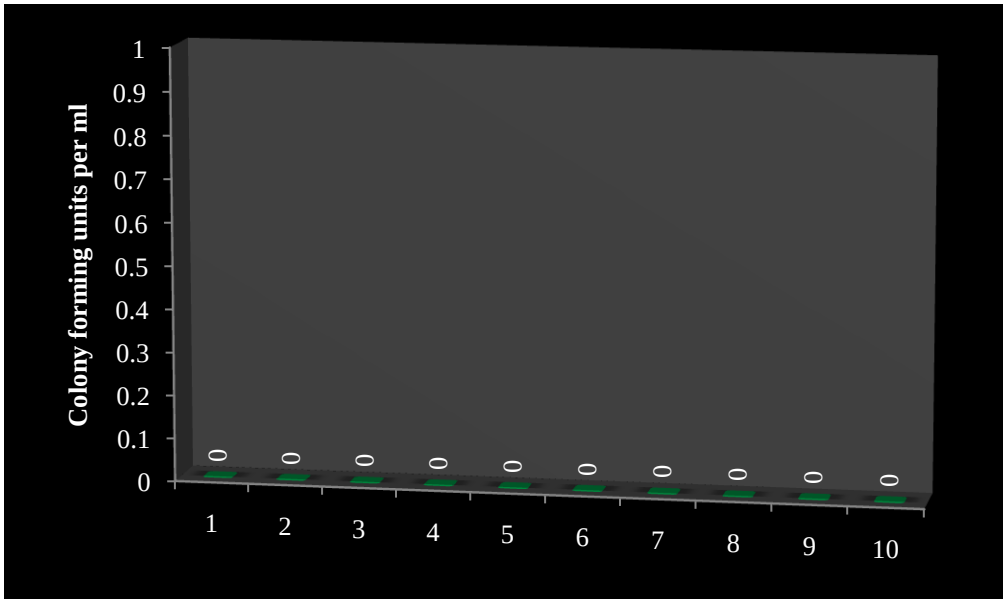
1b. Group II



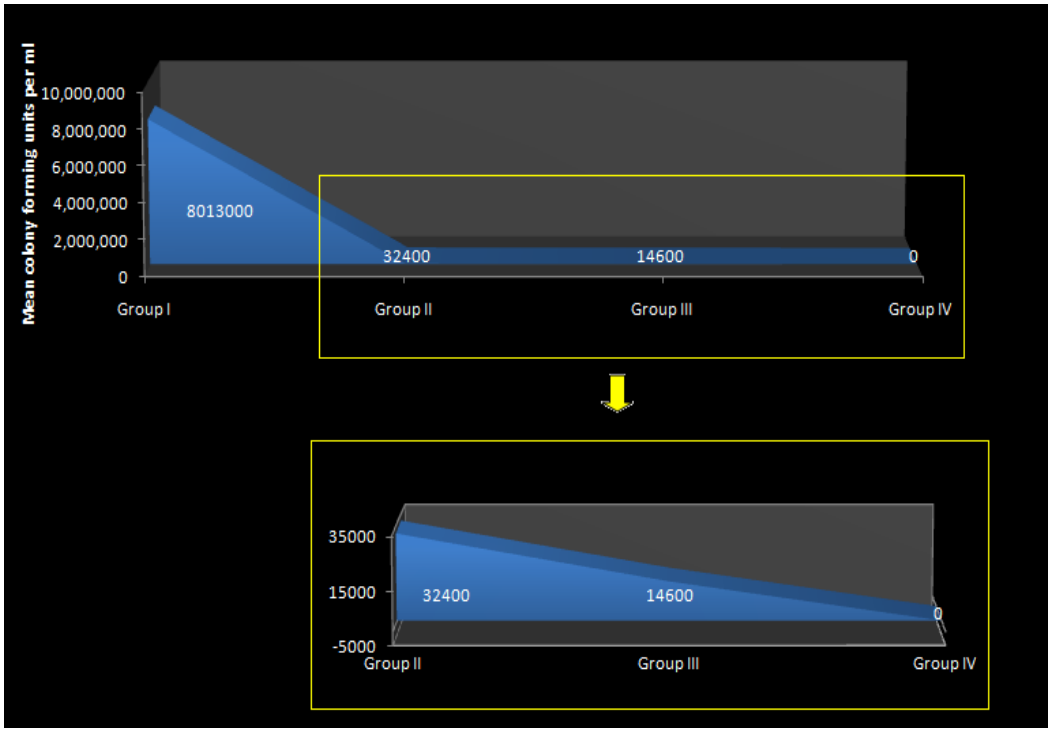
1c. Group III



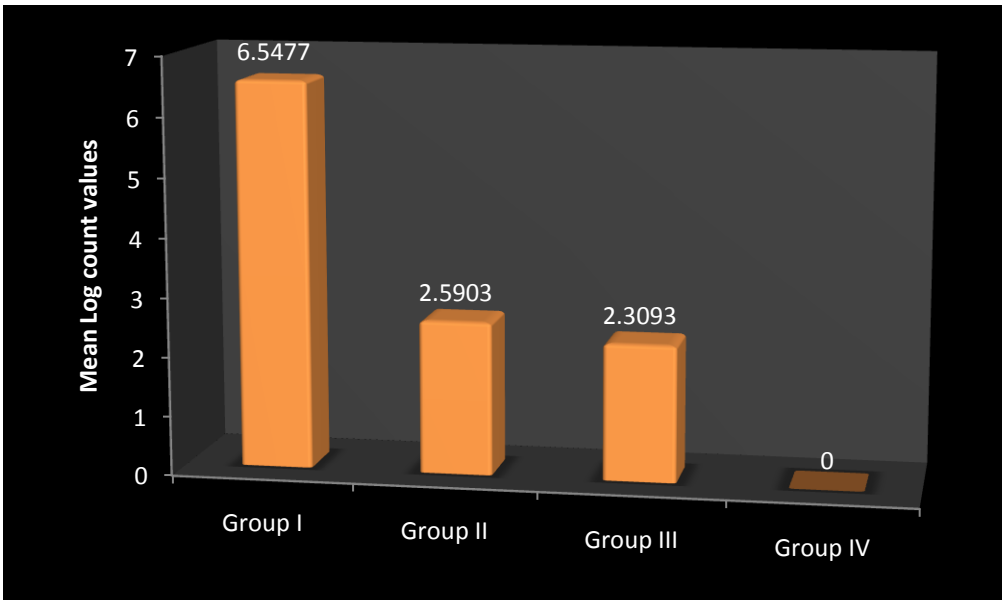
1d. Group IV



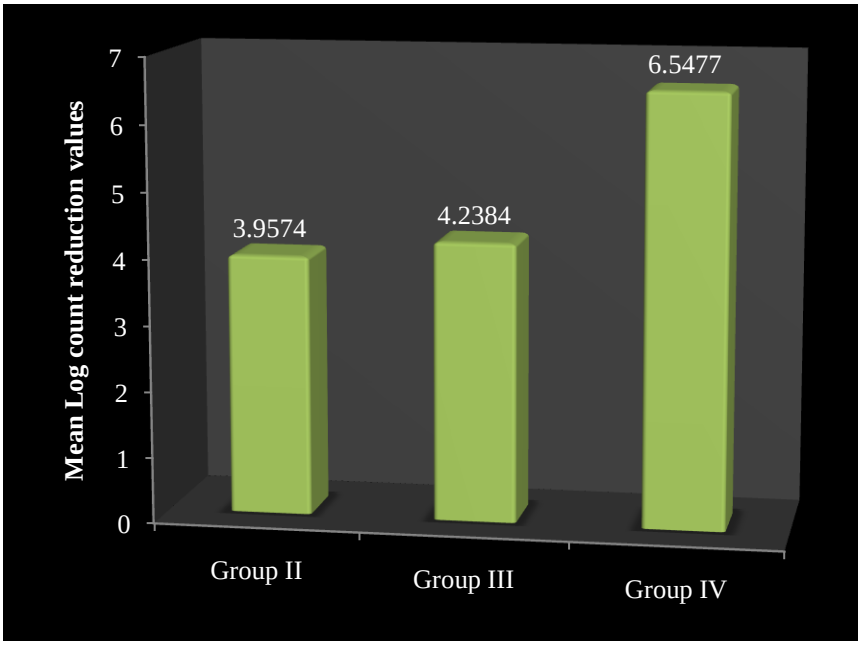
Graph 2: Mean value of colony forming units per ml (cfu/ml) obtained for Groups I, II, III and IV



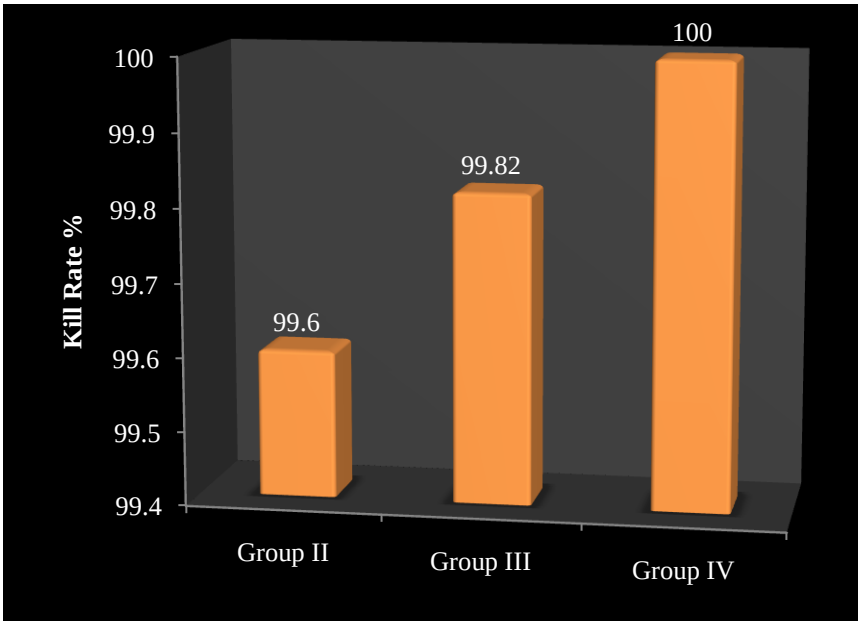
Graph 3: Mean log count ($\log_{10}$) values for Groups I, II, III and IV



Graph 4: Mean log count (log $_{10}$) reduction values for Groups II, III and IV

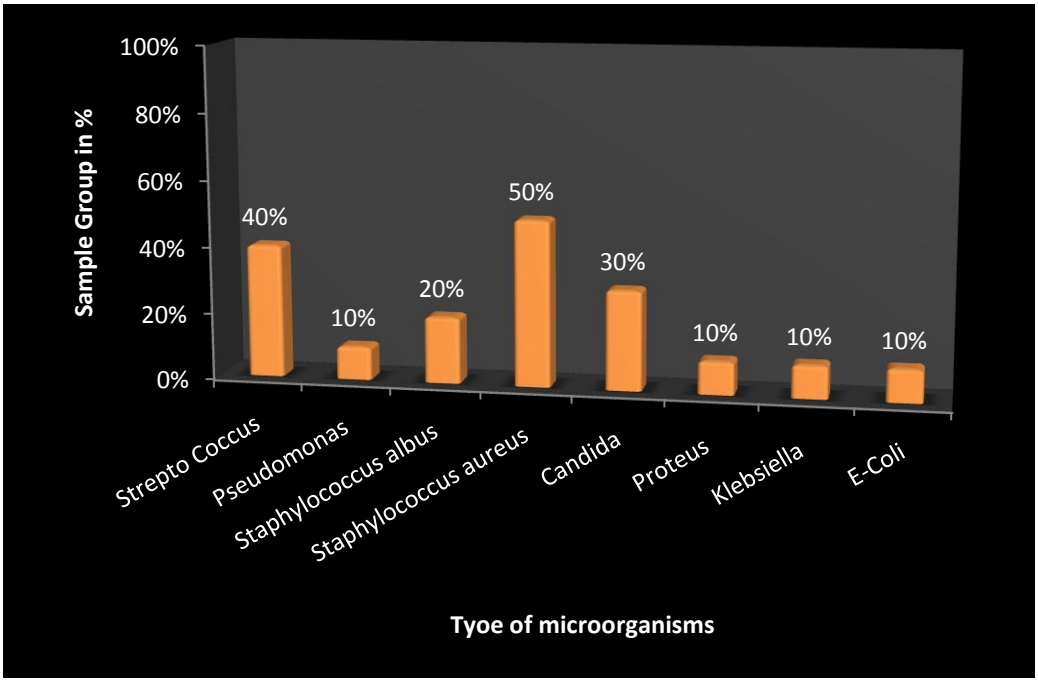


Graph 5: Kill rate % for Groups II, III and IV

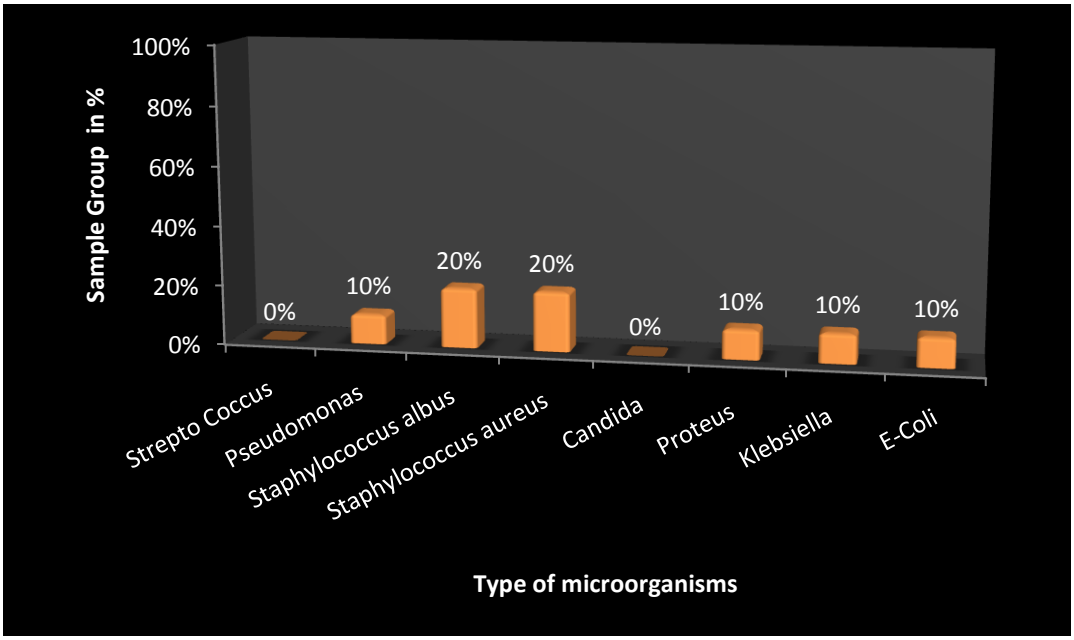


Graph 6: Isolation frequencies of microorganisms within Groups I, II and III

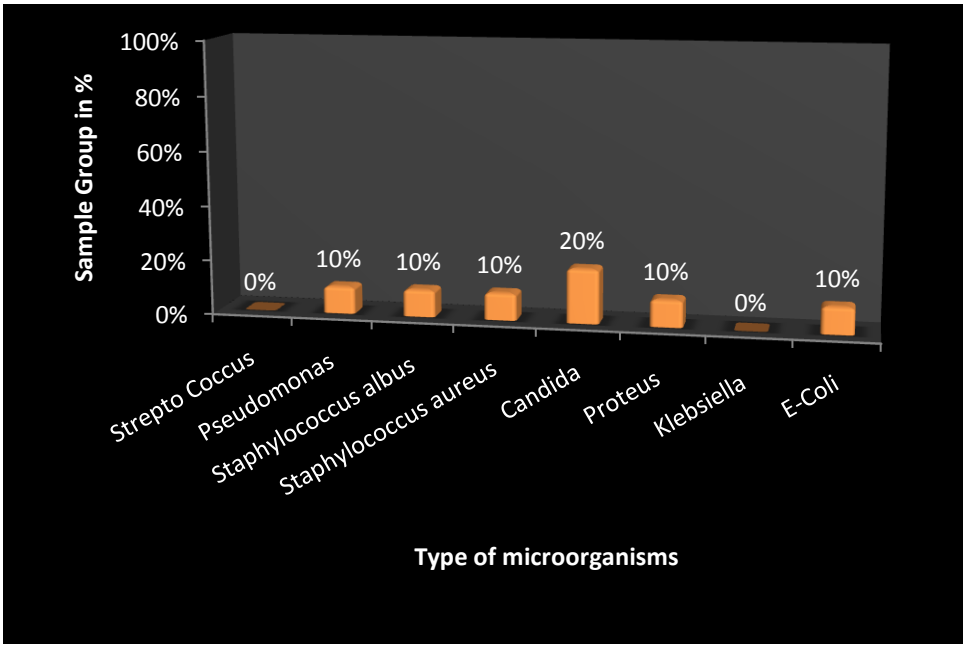
6a. Group I



6b. Group II

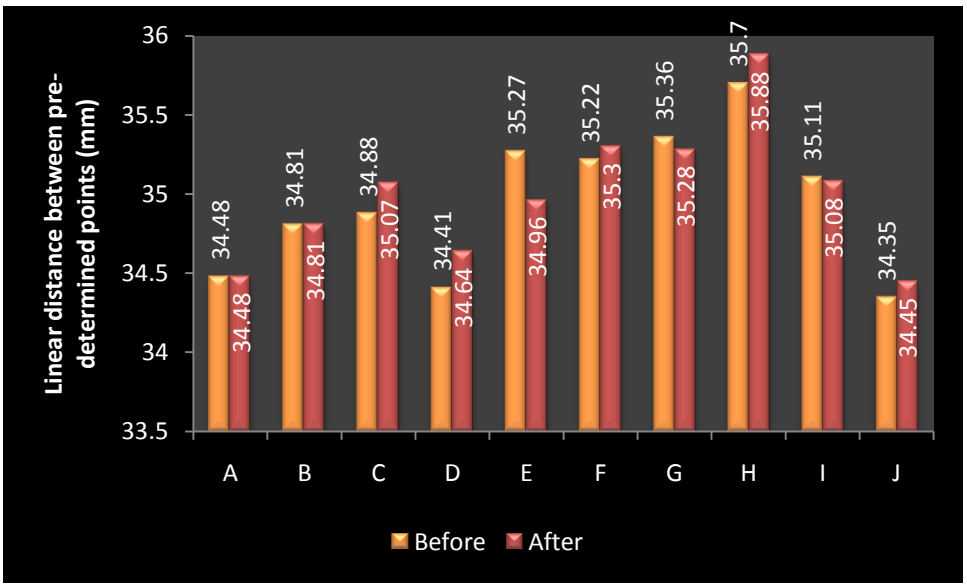


6c. Group III

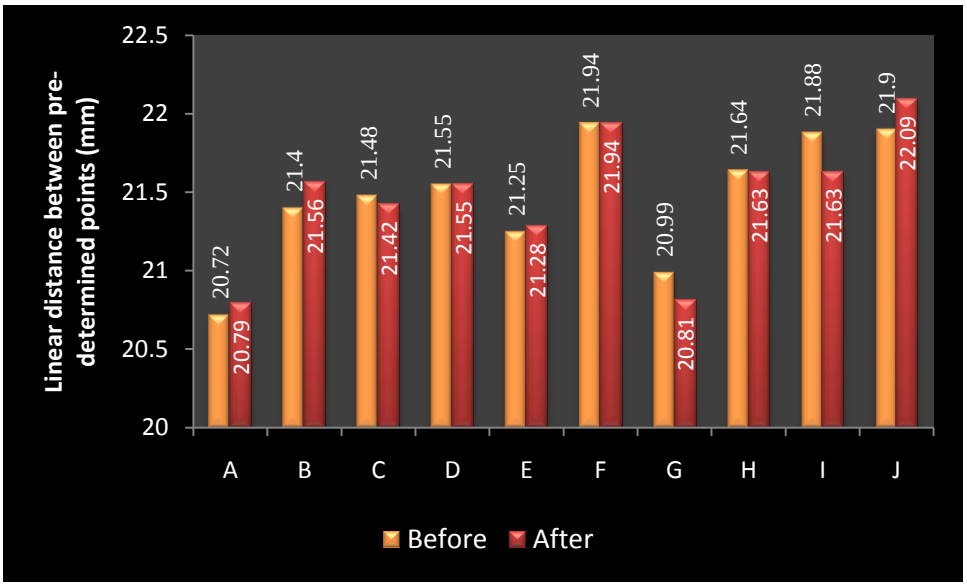

100%
80%
60%
40%
20%
0%
Sample Group in %
0%
10%
10%
10%
20%
10%
0%
10%
Strepto Coccus
Pseudomonas
Staphylococcus albus
Staphylococcus aureus
Candida
Proteus
Klebsiella
E-Coli
Type of microorganisms

Graph 7: Basic data of pre and post immersion linear measurements between predetermined reference points for Group V

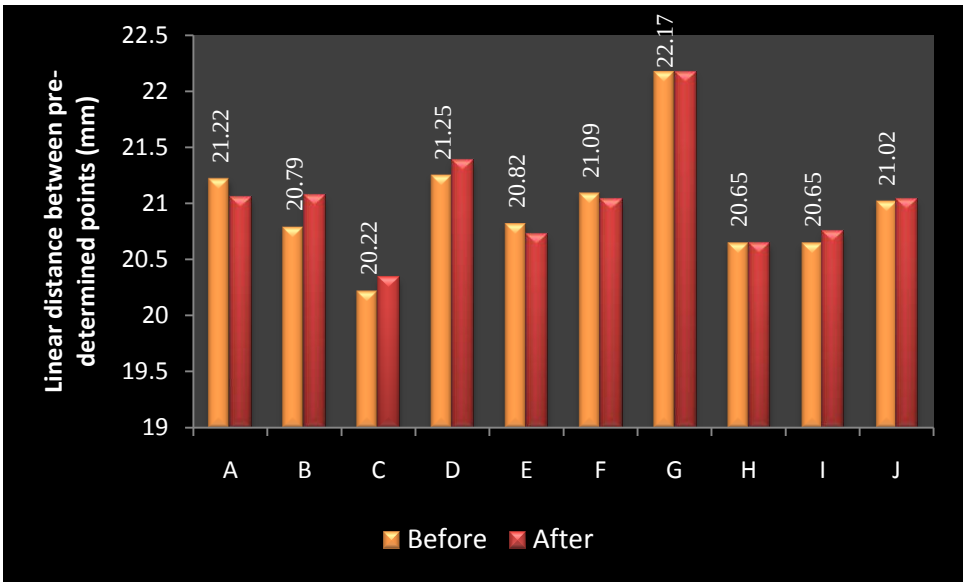
7a. Canine- Canine



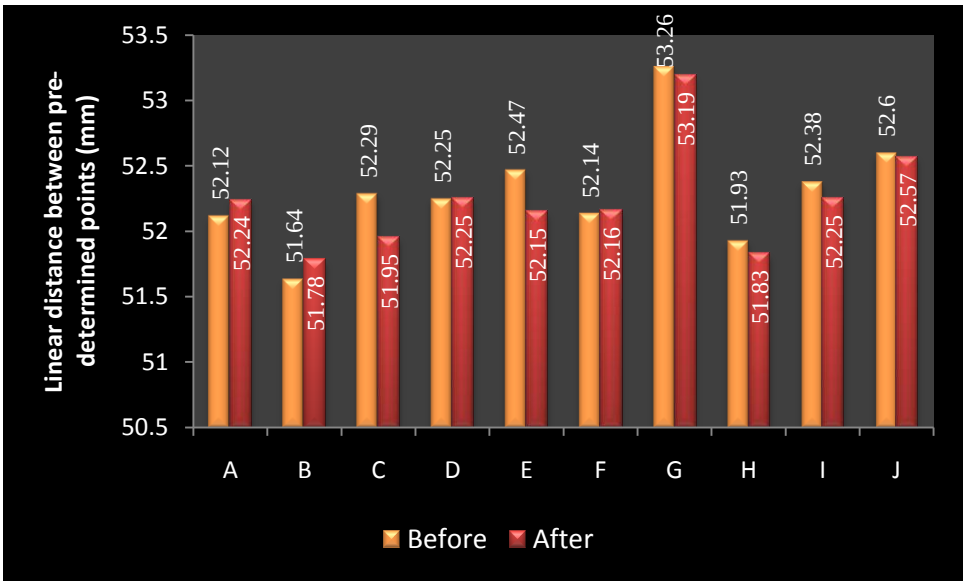
7b. Canine (Right) - Molar (Right)



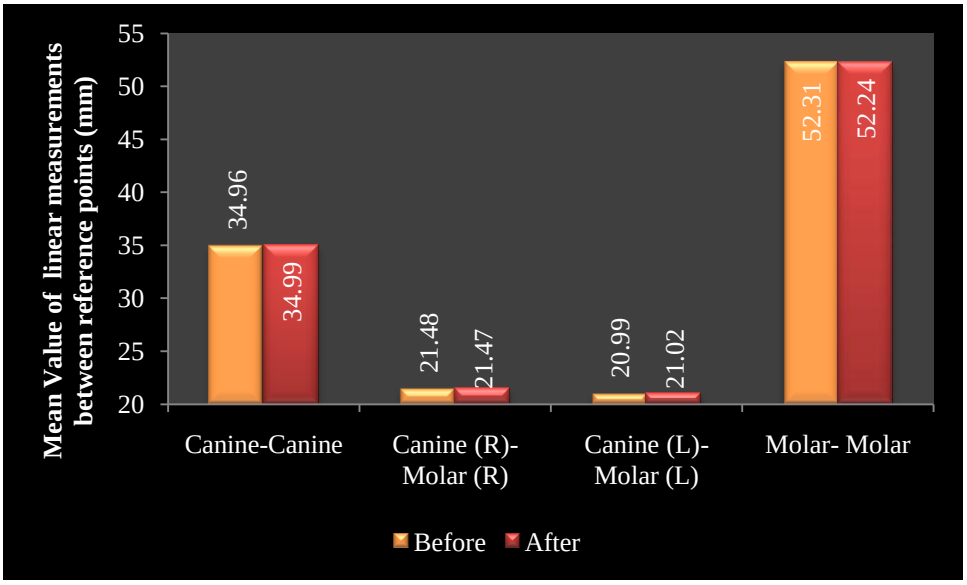
7c. Canine (Left) - Molar (Left)



7d. Molar- Molar

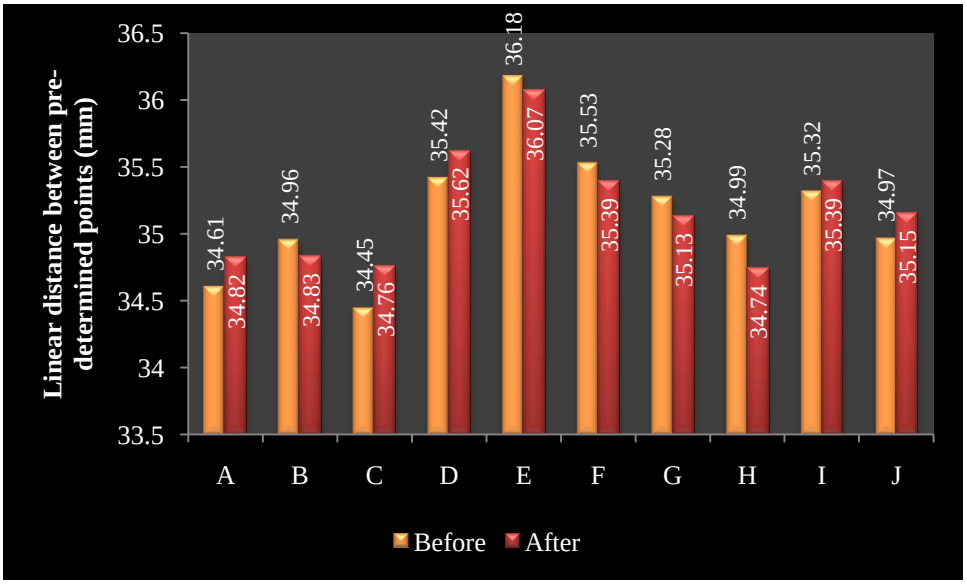


Graph 8: Mean values of pre and post immersion linear measurements between predetermined reference points for Group V

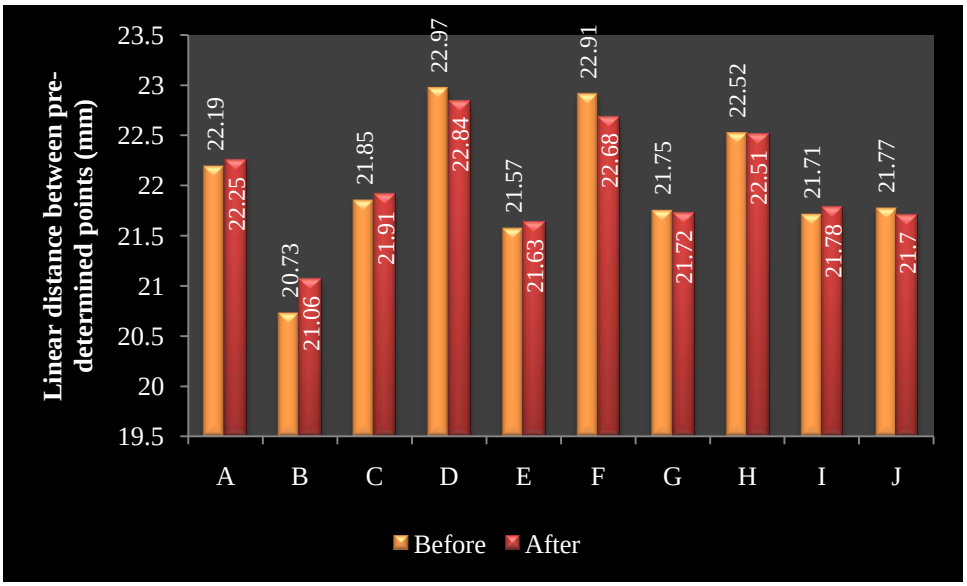


Graph 9: Basic data of pre and post immersion linear measurements between predetermined reference points for Group VI

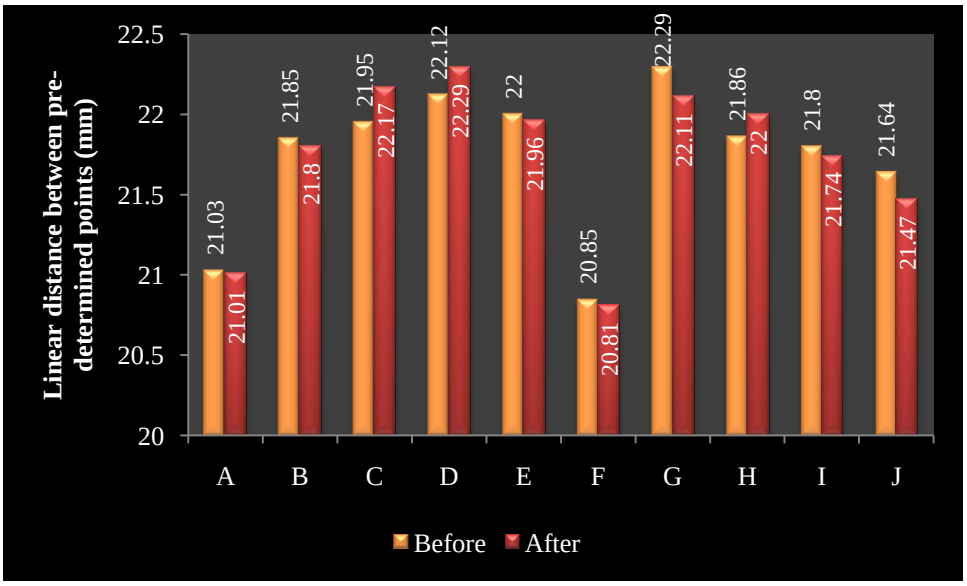
9 a. Canine- Canine



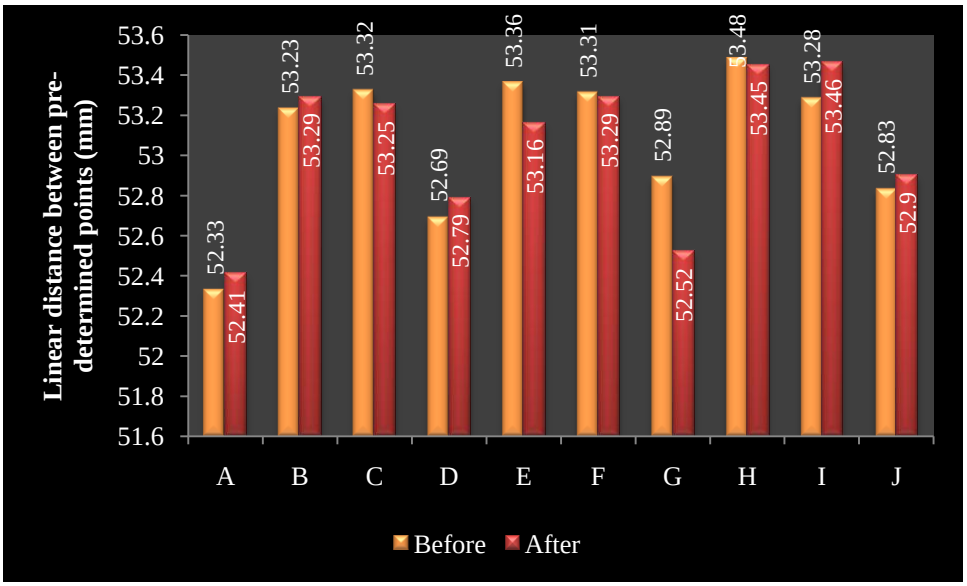
9 b. Canine (Right) - Molar (Right)



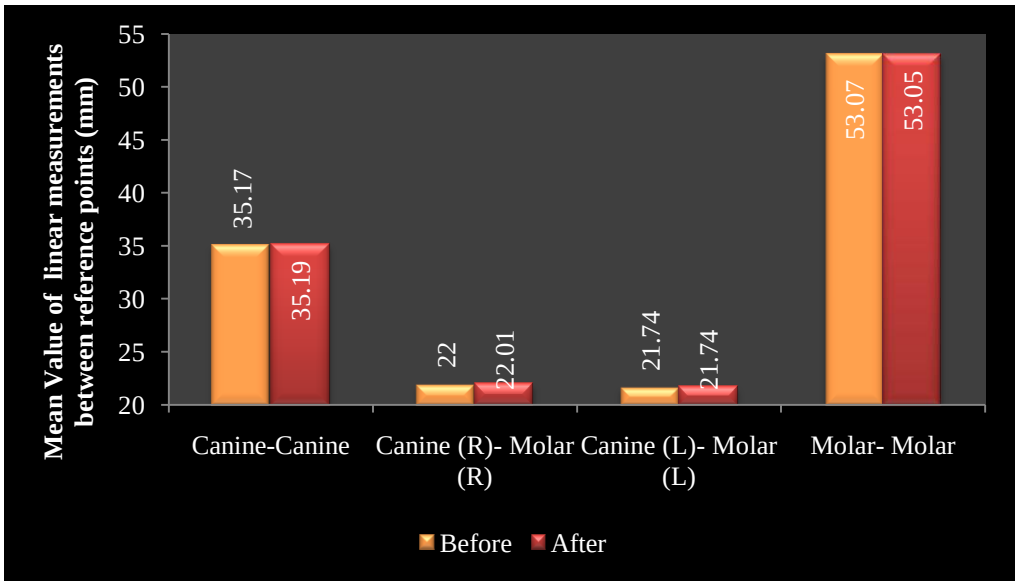
9 c. Canine (Left) - Molar (Left)



9 d. Molar - Molar

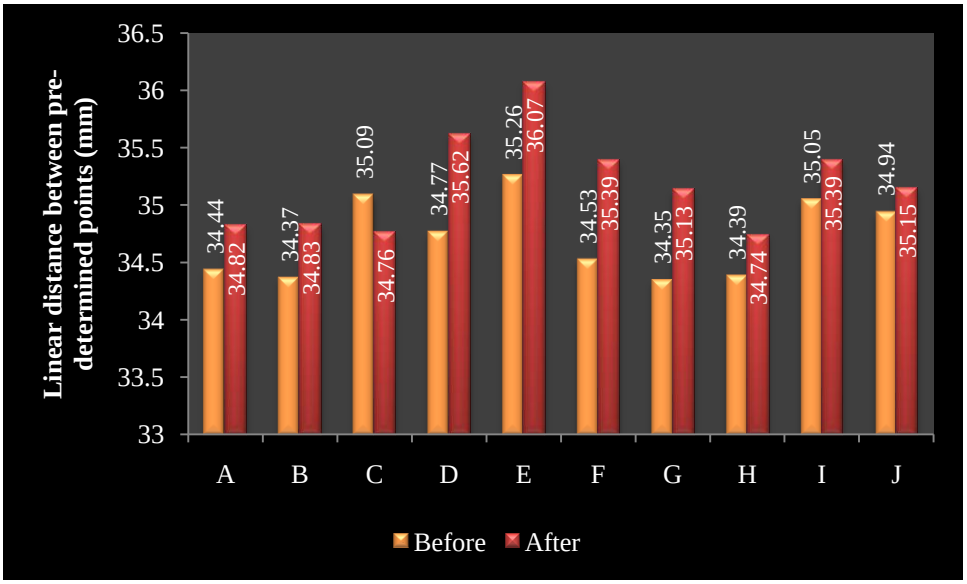


Graph 10: Mean values of pre and post immersion linear measurements between predetermined reference points for Group VI

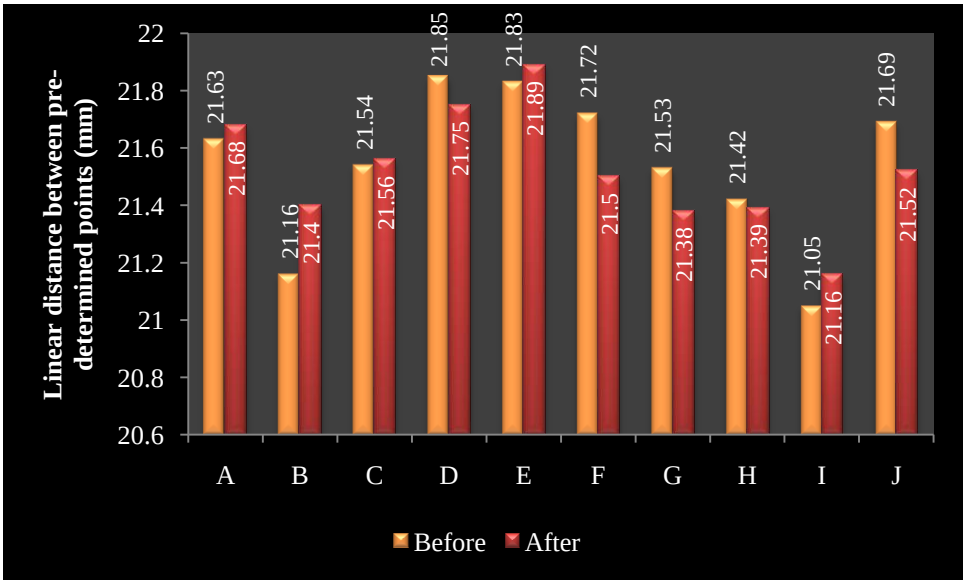


Graph 11: Basic data of pre and post immersion linear measurements between predetermined reference points for Group VII

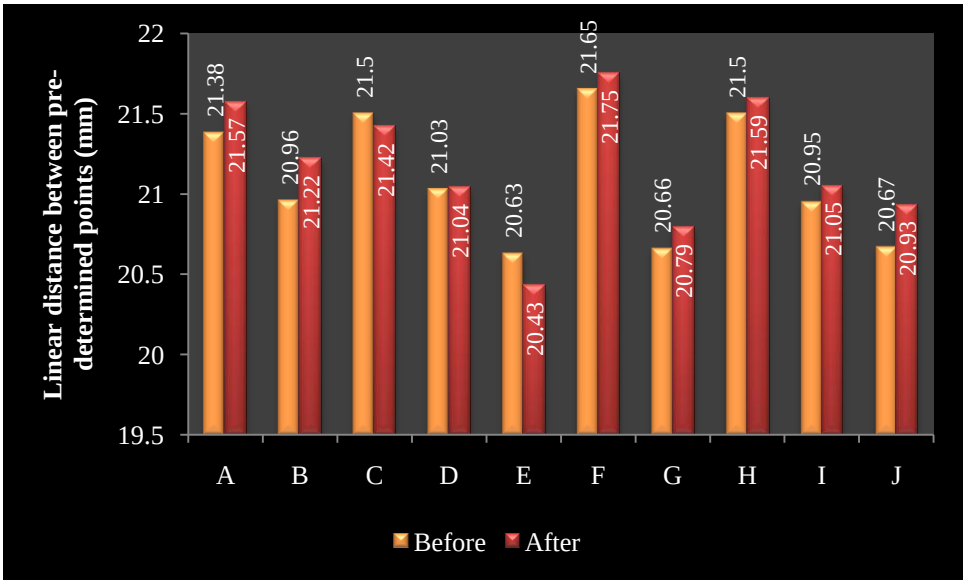
11a. Canine- Canine



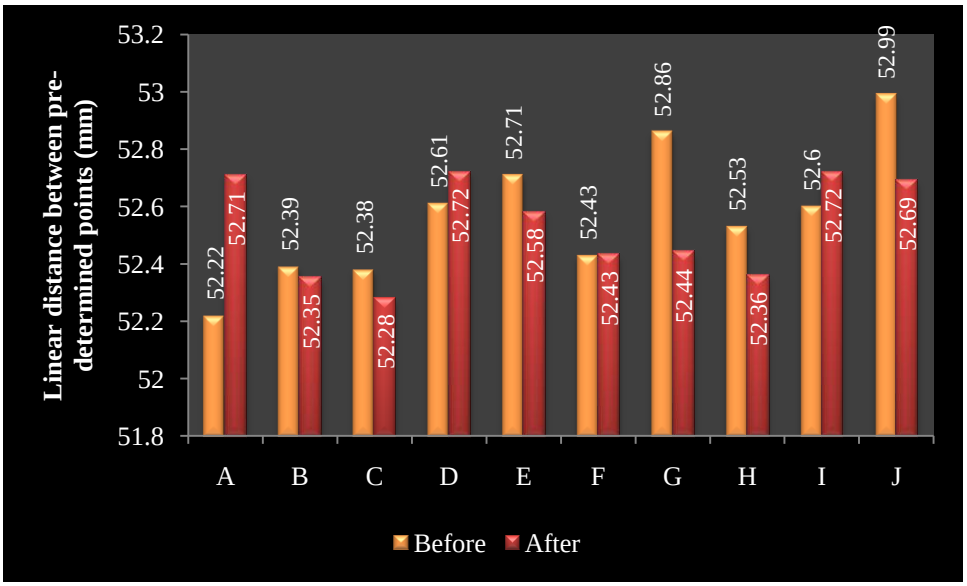
11b. Canine (Right) - Molar (Right)



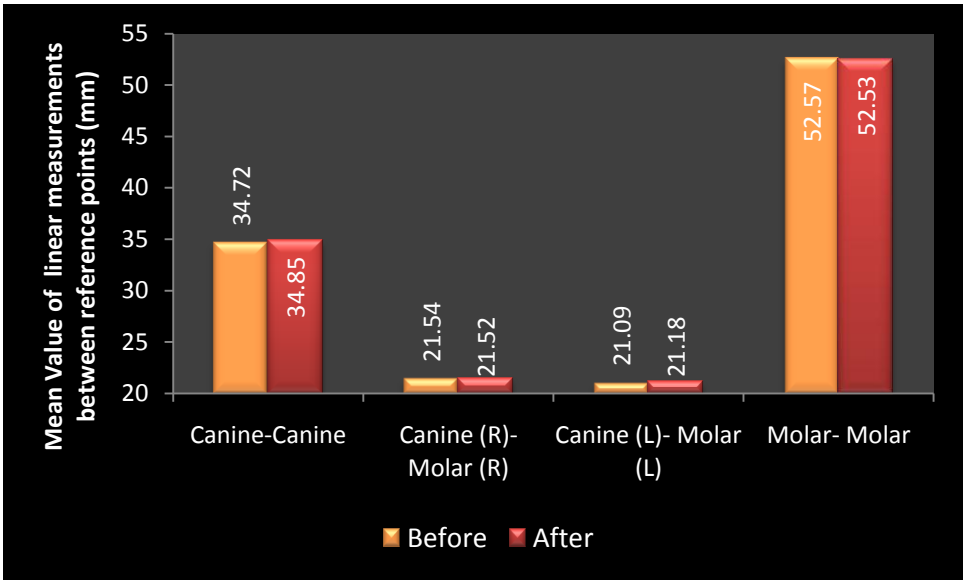
11c. Canine (Left) - Molar (Left)



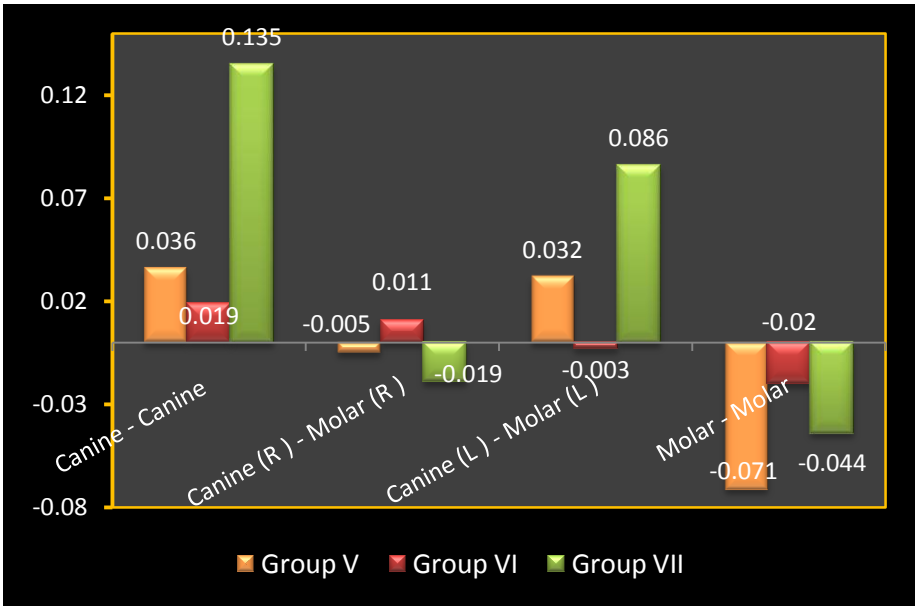
11d. Molar - Molar



Graph 12: Mean values of pre and post immersion linear measurements between predetermined reference points for Group VII



Graph13: Mean difference of pre and post immersion linear measurements between predetermined reference points for Groups V, VI and VII



DISCUSSION

Dental impressions are the stepping stones in obtaining well-fitting removable or fixed prostheses. Impression materials that come into contact with oral tissues, saliva and/or blood have been shown to act as fomite media for the potential transfer of organisms from patients to clinical and laboratory dental personnel, making them an essential topic of universal concern.[10, 16, 27]

The impressions made in the dental clinic for indirect fabrication of prosthesis are frequently sent to distant dental laboratories, usually enclosed inside plastic bags. This produces moisture conditions that are ideal for microbial survival and proliferation.[22]

It is insufficient to simply rinse the impressions with water without further disinfection procedures. It has been reported that washing the impressions with water alone removes only 40% of bacteria and should be regarded as merely a gross decontamination. On the other hand it has been suggested that impressions must be disinfected immediately after their removal from mouth without being rinsed or washed to reduce the risk of cross contamination.[4, 16]

Methods for disinfecting and sterilizing different types of dental impressions including those made with polyvinyl siloxane (PVS) have been reported. Disinfection measures include immersion or spraying of various chemical disinfectants while sterilization procedures include exposure to ethylene oxide gas, microwave, ultraviolet light and autoclaving.[2,4,7,16,20,22,24,29,46] These processes vary markedly in type, time and concentration.

Disinfection is generally a less lethal process than sterilization. It eliminates virtually all recognized pathogenic microorganisms, but not necessarily all microbial forms such as spores within 30 minutes. The disinfection by chemical disinfectants can be carried out in two ways: immersion or spraying. Immersion disinfection is more likely to expose all surfaces of the impressions to the disinfectant for the recommended time. Spraying disinfectant onto the surface of an impression reduces the chance of distortion but may not adequately cover the areas of undercut. [4,25,38,43]

However, no single method has been able to fulfill all requirements. For example, immersion in chemical solutions can compromise the dimensional accuracy of certain impressions; exposure to ultraviolet light does not produce a satisfactory antibacterial effect; and microwave is not suitable for impressions on metal trays. Similarly ethylene oxide gas sterilization for dental impressions is not a feasible option for all clinical settings. Autoclaving of impressions may eliminate all microbial contamination including spores, but is time consuming and not suitable for all impression materials.[63] Thus disinfection, rather than sterilization of dental impressions is a more practical approach on a day-today basis.

Among the various impression materials available for fixed tooth and implant supported prostheses, PVS impression material is the material of choice. This has been attributed to qualities such as best fine detail reproduction, elastic recovery, remarkable dimensional stability, wide variety of viscosities, rigidities

and working and setting times. Also they are odorless, tasteless and pleasant for patients.[11,13,42]

A variety of chemicals are available for the disinfection of dental impressions. However, not all impression materials are compatible with all types of disinfectants and some of these disinfectants may affect crucial properties of the impression materials such as, surface detail reproduction, surface roughness and dimensional stability.[4] The recommended and commonly used disinfectants for PVS impressions are glutaraldehyde and sodium hypochlorite.[6,12,37,44] Glutaraldehyde is a bactericidal, virucidal and fungicidal that is an effective disinfectant for PVS impressions. Further, glutaraldehyde is classified as a "high-level disinfectant" which may not inactivate spores but will destroy other microbes, in particular tubercle bacilli, HIV and HBV, by acting as a fixative reagent against proteins.[10, 16]

1% Sodium hypochlorite is an "intermediate-level disinfectant" which may not inactivate spores but will destroy other microbes, in particular tubercle bacilli, HIV and HBV. Hypochlorous acid, a substance present in sodium hypochlorite solution, when in contact with organic tissue acts as solvent, releases chlorine that combined with the protein amino group, forms chloramines. Hypochlorous acid (HOCl-) and hypochlorite ions (OCl-) lead to amino acid degradation and hydrolysis, that interferes in cell metabolism. Chlorine (strong oxidant) presents antimicrobial action inhibiting essential bacterial enzymes.[16, 17, 58]

Many studies have shown that PVS impressions can be successfully disinfected if they are immersed in 2% glutaraldehyde or 1% sodium hypochlorite.[3, 5,7,10,16,36,63]

Electrolyzed oxidizing water (EOW) is the product of a new concept developed in Japan. Research has revealed that electrolysis of deionized water containing a low concentration of sodium chloride (0.1%) in an electrolysis chamber where the anode and cathode electrodes are separated by a diaphragm, imparted strong bactericidal and virucidal properties to the water collected from the anode (EO water) .The theoretical sequence of chemical reactions involved in the production of EO water can be summarized as follows:

During electrolysis, sodium chloride dissolved in deionized water in the electrolysis chamber dissociates into negatively charged chloride (Cl^-) and hydroxy (OH^-) ions and positively charged sodium (Na^+) and hydrogen (H^+) ions. The chloride and hydroxy ions are adsorbed to the anode, with each ion releasing an electron (e^-) to become a radical. The chloric and hydroxy radicals combine, forming hypochlorous acid (HOCl), which separates from the anode. Two chloric radicals can also combine to produce chlorine gas. In the cathode section, each positively charged sodium ion receives an electron and becomes metallic sodium. The metallic sodium combines with water molecules, forming sodium hydroxide and hydrogen gas.[61]

The microbicidal properties of anode EOW are determined by its physical and chemical properties, such as low pH (3), high ORP (+1100 mV), and large concentrations of chlorine. Water from the anode normally has a pH of 2.7 or

lower, an oxidation-reduction potential (ORP) greater than 1,100 mV, and a free-chlorine concentration of 10 to 80 ppm.[19, 61, 63]

In aqueous solutions, Cl_2 hydrolyses rapidly into hypochlorous acid (HOCl). HOCl is one of the most germicidal chlorine compounds and is considered the chief factor in the disinfecting efficacy of EOW. Because of the water-like structure, the low molecular weight, and the electrical neutrality, HOCl molecules can easily diffuse through the bacterial cell wall into the cytoplasm and destroy it. EOW is easy to collect and poses no harm to personnel or the environment because it ultimately reverts to NaCl solution and can be produced with tap water and sodium chloride. However, EOW is effective only as a freshly prepared solution as it loses efficacy over time (after 24 hours).[18,19,61,63]

A complete inactivation of Candida albicans was obtained by a 5 minutes exposure to 5% EOW (20.0 mg/l free chlorine) and Staphylococcus aureus decreased to undetectable levels after 5 minutes of exposure to 7.5% anode EOW (30.0 mg/l Cl2) in a study and hence widely used in the food, medical, veterinary medicine and poultry industries as a disinfectant agent due to its wide bactericidal and fungicidal activity. Several hospitals in Japan routinely use EO water for surface sterilizing and hand washing and for treating wounds or disinfecting equipment.[9,19,61,63]

EOW as a potential disinfectant for hydrocolloid impressions, gypsum casts and titanium samples was investigated in a recent in-vitro study. Both immersion and ultrasonic nebulization methods for EOW application were tried. The results showed that a 10 minute immersion in EOW resulted in a 100% kill

rate.[63] The anti microbial efficacy of EOW on patient-derived PVS impressions has not been studied.

An undistorted impression is important to the fit of the future prostheses. Hence, dimensional stability of impression after being subjected to disinfection and other handling procedures is among the key desirable properties of an impression material. The dimensional stability of an impression material reflects its ability to maintain the accuracy of the impression over time.[55] Hence disinfection procedures that provide adequate antimicrobial efficacy without affecting the changes in impression dimensions are the focus of researchers.[31]

PVS impression materials seem to be relatively unaffected dimensionally by immersion in disinfectants such as glutaraldehyde and sodium hypochlorite.[5,14,23,25,34,36] The effect of immersion in EOW on the dimensional stability of hydrocolloid impressions showed significant changes.[63] However their effect on the dimensional stability of PVS impressions has not been studied.

In light of the above literature, the present study was aimed to comparatively evaluate the antimicrobial efficacy of three different chemical disinfectants and their effect on the dimensional stability of PVS impressions. The disinfectants employed were commercially available 2.4% glutaraldehyde, 1% sodium hypochlorite and freshly prepared EOW. The EOW was obtained with customized specifications (50mg/l free chlorine, with pH of 2.5, and ORP of 1150mv) based on a previous study and used within 24 hours.[18,63]

Studies regarding antimicrobial efficacy of glutaraldehyde and sodium hypochlorite are based primarily on the verification by in-vitro studies using test

microorganisms. The effects of disinfectants on artificially contaminated impressions (in-vitro) may differ from those on patient derived impressions (in-vivo) because of the presence of salivary and serum proteins on the impression surface or individual differences in oral flora composition. Clinical studies on the carriage of oral microorganisms onto the impression surface and the efficacy of disinfectants in removing them from patient-derived impressions are few. Hence, clinical study-based conclusive recommendations for disinfection procedures of dental impressions are also few.[15,16] Hence in the present study, the antimicrobial efficacy of the test disinfectant agents was studied on patient-derived PVS impression samples.

In this study, dentulous patients were selected based on previous studies that have stated that the microbial load on impression materials for such patients is significantly greater than that for edentulous patients. Proportionately fewer bacteria are retained on edentulous impressions than on impressions from dentate patients, due to abundant availability of ecologic arches in dentate mouths such as tooth surfaces, gingival crevices as opposed to edentulous patients with an oral flora restricted to mucosal surfaces. In the present study, dentate patients, who had not received any form of oral hygiene measures or therapeutic agents, were selected to avoid loss/suppression of oral microbial flora. Complete maxillary arch impressions were made in-line with the previous studies due to wider surface area available for better sampling.[4,10,54]

There is risk of removing plaque and associated microorganisms, if subsequent impressions were taken in short intervals, which may yield false

results. To eliminate this risk, previous studies had given a gap of 72 hours to two weeks between successive impressions. Considering this, in the present study, four impressions were made for each of the ten patients randomly on four different days with the time interval not less than 72 hours.[4,10]

As suggested in previous studies, to standardize the sterile protocol in this study, a PVS impression of the maxillary arch was made of a sterilized dental model and subjected to culture. Absence of live colonies indicated the adequacy of the sterilization procedures and acted as the negative control.[15,16]

Different protocols regarding rinsing of impressions prior to and after disinfection are available. Before any disinfection procedure is carried out, a thorough rinsing of the impression in tap water is recommended to remove blood, saliva and debris that may prevent exposure of the impression surface to the disinfectant. However, there is also a view that thorough rinsing of the impression prior to disinfection might alter the microbial load and have recommended a gentle rinsing in sterile or distilled water or no rinsing at all. In the present study, the impressions were subjected to gentle rinsing with distilled water for 45 seconds. Rinsing after disinfection is usually recommended to remove residual disinfectants that may affect the surface of the stone cast. Al-Jabrah et al in a similar study have stated that such procedures may remove microorganisms that have survived the disinfection procedures thereby affecting the results. Based on the latter view, impressions were not subjected to rinsing after disinfection in the present study.[4,7]

The present study used BHI agar for microbial culture as it is an enriched nonselective medium. It allowed a large number of varied colonies to grow in the present study, facilitated visualization of the bacterial colonies and their distribution on the culture surface. Further plating on selective agar plates for selective isolation of oral microorganisms was done. Sabourads agar was used for isolation of Candida and blood agar for isolation of streptococci. Organisms were also confirmed by gram staining and biochemical tests.[10,15,16]

There are number of different methods to determine the number of micro-organisms (colony forming units) that are present in a given population, such as the spectrophotometer to measure the optical density of the population, direct counting of the microorganisms using a haemocytometer, or by serial diluting the bacteria and plating the diluted bacteria on media that supports the growth of the micro-organisms. The latter method is more time consuming, but provides statistically accurate and repeatable results and was adopted in this study.[27]

The antimicrobial efficacy of the three test disinfectants in this study were quantitatively obtained by calculating the $\log_{10}$ count reduction and kill rate%. These parameters were chosen based on previous study.[63] The data obtained was tabulated and statistically analyzed. The qualitative analysis of the type and isolation frequencies of the microbial flora was also done.

For determining the dimensional stability of polyvinyl siloxane impressions subjected to three test disinfectants, a maxillary dental model with typhodont teeth was chosen. This was done to maintain uniformity of all samples obtained and also to avoid subjecting patients to additional impression making

procedures and patient-related variables. The dimensional stability of PVS impressions has been indirectly measured on test dies in accordance with ADA specification 19 [2,5,24,32,38,49] or on full arch casts. [1,3,25,36,52] Few studies have directly measured dimensional changes on impressions. Direct evaluation of full arch impressions has been recommended to avoid errors associated with cast pouring which was followed in the present study.[34,60]

Various authors have employed different measuring equipments and techniques in determining dimensional changes before and after disinfection procedures. These include using a Boley's gauge[39], measuring microscope [25,30,36,56,57,59], digimatic calipers[58], toolmakers microscope[24], Nikon profile projector[3], Nikon measurescope[14,62] and 2D computer scanner [5].Previous studies have shown that PVS impression material exhibits superior dimensional stability when compared to polyether and irreversible hydrocolloids. Technological advances have made possible the use of more sophisticated and accurate methods of assessing the dimensional stability of dental materials. This becomes more relevant when measuring dimensional changes of a more stable material like PVS.

In a recent study by Kollefrath, impressions were subjected to CT scanning and dimensional changes calculated by overlay of the scans. CT scanning captures the precise details of an impression and has sufficient accuracy for direct measurement of impressions.[28,29] CT scanned images reconstructed by Mimics software has been employed for obtaining accurate anthropometrical measurements. Studies have shown that linear measurements done on CT scanned

images are reliable and accurate as computerized analysis may result in a significant reduction of measurement errors.[8,47] In view of the above, in the present study, PVS impressions were directly subjected to CT scanning and reconstructed using Mimics software for obtaining 3D virtual images for conducting linear measurements. Since impressions had to be subjected to CT procedures, polycarbonate impression trays were used instead of metal trays as the latter are not suitable for this purpose.[28]

Metal balls, 4mm in diameter were placed on the impressions at four predetermined reference points to provide accurate points of reference and to facilitate the measurement of distance between them.[28] The mode used for obtaining the CT images was an extensive reconstruction scale, which helps obtaining fine images despite presence of metal balls. The reference points chosen for measuring the dimensional changes in full arch samples varied in previous studies.[3,36,57,58] The reference points used in the present study were the cusp tip of right and left canines and mesiobuccal cusp tip of right and left first molar and these points were easily recognized in all the impressions, and were chosen based on previous studies.[8,26, 60]

Linear measurements were done between the centers of the metal balls placed at the above determined points to standardize the measurement procedure for all the impressions.[28] The distances measured were the inter-canine distance, the right and left canine to the respective right and left molar distances and the inter-molar distance. All measurements for all the samples were done by a single

operator to avoid errors and the results obtained were tabulated and statistically analyzed. The results obtained can be discussed under the following heads:

1. Antimicrobial efficacy of three different chemical disinfectants on patient-derived PVS impressions.
2. Effect of the three different chemical disinfectants on dimensional stability of dental model-derived PVS impressions.

1. Antimicrobial efficacy of three different chemical disinfectants on patient-derived PVS impressions:

The results obtained were tabulated as colony forming units per ml for all the groups. All the samples in Group I (control group) showed the presence of microbial flora with a mean colony forming units per ml of 8013000.0000 (cfu/ml) (Table 2) and Streptococcus(40%), Staphylococcus aureus(50%), Staphylococcus albus(20%), Klebsiella(10%), Candida albicans(30%), E.coli(10%), Proteus(10%) and Pseudomonas aeruginosa (green pigment) (10%) were detected .

60% samples in Group II (2.4% glutaraldehyde) showed the presence of microbial flora with a mean colony forming units per ml of 32400.0000 (cfu/ml) (Table 2) and Staphylococcus aureus (20%), Staphylococcus albus (20%), Klebsiella (10%), E.coli (10%) and Pseudomonas aeruginosa (green pigment) (10%) were detected.

60% samples in Group III (1% sodium hypochlorite) showed the presence of microbial flora with a mean colony forming units per ml of 14600.0000(cfu/ml)

(Table 2) and Staphylococcus aureus(10%), Staphylococcus albus(10%), Candida albicans (20%), E.coli (20%) and Proteus(10%) were detected .

All samples in Group IV (freshly prepared electrolyzed oxidizing water) showed no microbial growth with a mean colony forming unit of 0.0000 cfu/ml (Table 2).

The colony forming units were transformed in to $\log_{10}$ count values and the results presented as the mean of $\log_{10}$ count values for each group. The mean $\log_{10}$ count value for Groups I, II, III and IV were 6.5477, 2.5903, 2.3093 and 0.0000 respectively (Table 3). These values were found to be significantly different from each other statistically.

Previous studies have shown that relatively fewer microorganisms adhere to PVS impressions as compared to hydrocolloid impressions. [10, 54] In the present study only PVS impressions were tested and all the control specimens exhibited microbial growth. The mean colony forming units per ml (8013000.0000) and mean $\log_{10}$ count values (6.5477) were also high for this group underlining the importance of decontaminating all such impressions prior to further handling.

The $\log_{10}$ count reduction values were obtained by calculating the difference between the $\log_{10}$ count values of the control group and the disinfectant group. The mean $\log_{10}$ count reduction for Groups II, III & IV was 3.9574, 4.2384 and 6.5477 respectively (Table 4). The kill rate (%) for Group II was 99.60%, Group III was 99.82 % and Group IV was 100% (Table 6).

On comparison the mean log_{10} count reduction value for the three test groups were found to be statistically different. Group III showed a mean log_{10} count reduction value which was slightly higher than that obtained for Group II. However this increase in reduction value was statistically insignificant. Group IV showed the highest log reduction value which was statistically significant than both Groups II and III (Table 5).

A study by Bustos J et al revealed that patient-derived silicone specimens showed complete elimination of bacteria after being subjected to 2%glutaraldehyde and 0.5% sodium hypochlorite for 10 min. Wu G., et al compared the effect of sodium hypochlorite and freshly prepared EOW(by immersion and nebulisation) on the microbial flora and dimensional stability of hydrocolloid impressions. Their results showed a log_{10} reduction of microbial colonies for sodium hypochlorite to be around 4, and that for immersion in EOW around 6. The kill rate% for immersion in sodium hypochlorite was found to be lower than that for EOW which exhibited 100 % kill rate. [10, 63] The findings in the present study are in line with the above studies, with both glutaraldehyde and sodium hypochlorite exhibiting similar antimicrobial efficacy with similar log count reduction values of 3.9574 and 4.2384 and kill rate % of 99.60% and 99.82% respectively and EOW exhibiting highest log_{10} count reduction value greater than 6 which was statistically significant and a kill rate % of 100%. A log count reduction value of around four is considered as the gold standard for dental disinfectant. All the three disinfectants tested showed a log_{10} reduction value of 4 and above and a kill rate % of above 99 which is considered as acceptable antimicrobial efficacy for a dental disinfectant.

The type of microorganisms isolated in the present study as mentioned above are predominantly similar to those observed in previous in-vivo studies with few differences in the type and isolation frequencies.[7, 10,15,16,48,54] This could be attributed to variations in test sample populations and culturing techniques.

2. Effect of three different chemical disinfectants on dimensional stability of dental model- derived PVS impressions:

The data obtained from the linear measurements for all the samples were tabulated and the mean for each set of measurements obtained and subjected to statistical analysis.

The means of linear measurements on pre and post immersion 3D images of Group V between predetermined reference points was found to be 34.96mm & 34.99mm for canine-canine, 21.48mm & 21.47mm for canine(R)-molar(R), 20.99mm &21.02mm for canine(L)-molar(L) and 52.31mm and 52.24mm for molar-molar measurements respectively (Table 8).

The means of linear measurements on pre and post immersion 3D images of Group VI between predetermined reference points was found to be 35.17mm & 35.19mm for canine-canine, 22.00mm &22.01mm for canine(R)-molar(R), 21.74mm &21.74mm for canine(L)-molar(L) and 53.07mm and 53.05mm for molar-molar measurements respectively (Table 10).

The means of linear measurements on pre and post immersion 3D images of Group VII between predetermined reference points was found to be 34.72mm & 34.85mm for canine-canine, 21.54mm &21.52mm for canine(R)-molar(R),

21.09mm &21.18mm for canine(L)-molar(L) and 52.57mm and 52.53mm for molar-molar measurements respectively (Table 12).

The differences between the means of pre and post immersion measurements within Groups V, VI&VII respectively were found to be statistically insignificant.

The mean pre and post immersion dimensional differences as measured between canine-canine for Groups V, VI&VII were found to be 0.0360, 0.0190, and 0.1350 respectively (Table 13). In canine-canine region, all specimens of 2.4% glutaraldehyde, 1% sodium hypochlorite and freshly prepared electrolyzed oxidizing water exhibited a slight expansion between pre and post immersion linear measurements.

The mean pre and post immersion dimensional differences as measured between canine (R)-molar(R) for Groups V, VI and VII were found to be -0.0050, 0.0110 and -0.0190 respectively (Table 13). In the canine(R)-molar(R) region, all specimens of 2.4% glutaraldehyde and freshly prepared EOW exhibited a slight shrinkage between pre and post immersion linear measurements, whereas all specimens of 1%sodium hypochlorite exhibited a slight expansion.

The mean pre and post immersion dimensional differences as measured between canine (L)-molar (L) for Groups V, VI and VII were found to be 0.0320, -0.0030 and 0.0860 respectively (Table 13). In the canine (L)-molar (L) region, all specimens of 2.4% glutaraldehyde and freshly prepared EOW exhibited a slight expansion, between pre and post immersion linear measurements whereas, all specimens of 1%sodium hypochlorite exhibited a slight shrinkage.

The mean pre and post immersion dimensional differences as measured between molar-molar for Groups V, VI and VII were found to be -0.0710, -0.0200 and 0.0440 respectively (Table 13). In the molar-molar region, all specimens of 2.4% glutaraldehyde and 1% sodium hypochlorite exhibited a slight shrinkage between pre and post immersion linear measurements, whereas all specimens of freshly prepared EOW exhibited a slight expansion.

Though slight contractions or expansions were observed in the different regions measured, the mean differences for each region were found to be statistically insignificant when compared between the three groups (Groups V, VI& VII) (Table 14).

PVS impressions have been tested for dimensional stability either alone or in combination with the other impression materials by immersion for 10 minutes or more, in a number of chemical disinfectants including 2% glutaraldehyde and 1% sodium hypochlorite in previous studies. None of the disinfectant agents had caused any significant dimensional changes, indicating the overall superior dimensional stability of PVS impression material.[23, 24, 32, 34-36] The findings in the present study are in agreement with these studies regarding a 10 minutes immersion in 2.4 % glutaraldehyde and 1% sodium hypochlorite.

Wu G. et al in their study compared the dimensional stability of irreversible hydrocolloids by immersion in 1% sodium hypochlorite and freshly prepared EOW.[63] Immersion in 1% sodium hypochlorite and freshly prepared EOW caused significantly higher dimensional changes in their study. This can be attributed to the inherent properties of irreversible hydrocolloids. However, 10

minutes immersion in freshly prepared EOW in the present study caused negligible dimensional changes indicating the dimensional stability of PVS impression material. These findings reaffirm the superior dimensional stability of PVS impression material after immersion disinfection. The findings in the present study suggest that freshly prepared EOW is a potent disinfectant in addition to glutaraldehyde and sodium hypochlorite for PVS impressions without affecting dimensional stability.

The present study had certain limitations. The antimicrobial efficacy of the three test disinfectants was tested under clinical conditions against oral bacteria and fungi. Further research is needed to investigate the efficacy of these disinfectants, especially for EOW, against viruses and resistant bacterial species. The efficacy of electrolyzed oxidizing water as a spray disinfectant and its shelf life were was also not evaluated. A wider range of impression materials and ways to improve the culturing technique should also be investigated along with three dimensional evaluations for dimensional changes and tests for surface quality and detail reproduction to enhance the results obtained with the present study.

The results obtained in this study as well as previous studies that have described disinfection methods, suggests that it is prudent for dentists, dental auxiliaries and dental technicians to disinfect impressions with suitable disinfectants in order to prevent the transmission of diseases without affecting the dimensional stability.

CONCLUSION

The following conclusions were drawn from the data obtained in the present study, conducted to comparatively evaluate the antimicrobial efficacy of three different chemical disinfectants and their effect on the dimensional stability of polyvinyl siloxane (PVS) impressions.

1. The Group I **(Control – Untreated)**, patient-derived PVS impressions on microbial culture showed the presence of microbial flora with a mean colony forming units per ml (cfu/ml) value of 8013000 .0000 and a mean log count value of 6.5477.The type and isolation frequencies of micro organisms after culture were as follows: Pseudomonas aeruginosa (green pigment)(10%), Streptococcus (40%), Candida albicans (30%), Staphylococcus albus (20%), Staphylococcus aureus (50%), Proteus (10%), Klebsiella (10%) and E.Coli (10%).
2. The Group II, patient-derived PVS impressions (**10 minutes immersion in 2.4% Glutaraldehyde)** on microbial culture showed a gross reduction in microbial growth with a mean colony forming units per ml (cfu/ml) value of 32400.0000 and a mean log count value of 2.5903.

   The type and isolation frequencies of micro organisms after culture were as follows: Pseudomonas aeruginosa (green pigment) (10%), E.Coli (10%), Staphylococcus albus (20%), Proteus (10%), Staphylococcus aureus (20%) and Klebsiella (10%).
3. The Group III, patient-derived PVS impressions (**10 minutes immersion in 1 % Sodium Hypochlorite)** on microbial culture showed a gross reduction

in microbial growth with a mean colony forming units per ml (cfu/ml) value of 14600.0000 and a mean log count value of 2.3093.

The type and isolation frequencies of micro organisms after culture were as follows: Pseudomonas aeruginosa (green pigment) (10%), Candida albicans (20%), E.Coli (20%), Staphylococcus albus (10%), Proteus (10%) and Staphylococcus aureus (10%).

4. The Group IV, patient-derived PVS impressions (**10 minutes immersion in freshly prepared Electrolyzed oxidizing water)** on microbial culture showed no microbial growth with a mean colony forming units per ml (cfu/ml) value of 0.0000 and a mean log count value of 0.0000.
5. The antimicrobial efficacy (Mean log_{10} reduction and Kill rate %) of the three chemical disinfectants in the present study was as follows:
    - Group II samples showed a Mean log_{10} reduction of **3.9574** and **99.60%** kill rate.
    - Group III samples showed a Mean log_{10} reduction of **4.2384** and **99.82%** kill rate.
    - Group IV samples showed a Mean log_{10} reduction of **6.5477** and **100%** kill rate.
6. All the three disinfectant Groups (Groups II, III and IV) showed a statistically significant difference in the mean log_{10} reduction when compared with the control Group. (p-value 0.010**; highly significant).
7. The Group II and Group III specimens showed no statistically significant difference in their mean log_{10} reduction values. (p-value > 0.05; Insignificant).

Log_{10} count reduction of Group II = Log_{10} count reduction of Group III

8. The Group IV specimens showed a statistically significant difference in the mean log_{10} reduction value when compared to those of Group II and Group III (p-Value < 0.05; Significant).

   Log_{10} count reduction of Group IV > Log_{10} count reduction of Groups II and III

9. The means of linear measurements on pre and post immersion 3D images of Group V at predetermined reference points was found to be 34.96mm & 34.99mm for the canine-canine, 21.48mm & 21.47mm for the canine(R)-molar(R), 20.99mm & 21.02mm for the canine(L)- molar(L) and 52.31mm & 52.24mm for the first molar-first molar measurements respectively.

   The differences between the means of pre and post immersion measurements between the above predetermined reference points within Group V was found to be statistically insignificant (p-Value>0.05).

10. The means of linear measurements on pre and post immersion 3D images of Group VI at predetermined reference points was found to be 35.17mm & 35.19mm for the canine-canine, 22.00mm & 22.01mm for the canine(R)-molar(R), 21.74mm & 21.74mm for the canine(L)- molar(L) and 53.07mm & 53.05mm for the first molar-first molar measurements respectively.

    The differences between the means of pre and post immersion measurements between the above predetermined a reference point within Group VI was found to be statistically insignificant (p-Value > 0.05).

11. The means of linear measurements on pre and post immersion 3D images of Group VII at predetermined reference points was found to be 34.72mm & 34.85mm for the canine-canine, 21.54mm & 21.52mm for the canine(R)-molar(R), 21.09mm & 21.18mm for the canine(L)- molar(L) and 52.57mm & 52.53mm for the first molar-first molar measurements respectively.

    The differences between the means of pre and post immersion measurements between the above predetermined reference points within Group VII was found to be statistically insignificant (p-Value > 0.05).
12. The mean pre and post immersion dimensional differences as measured between canine-canine for Groups V, VI and VII were found to be 0.0360, 0.0190 and 0.1350 respectively. These differences were found to be statistically insignificant when compared between each other (p-value > 0.05).
13. The mean pre and post immersion dimensional differences as measured between Canine (Right) – Molar (Right) for Groups V, VI and VII were found to be -0.0050, 0.0110 and -0.0190 respectively. These differences were found to be statistically insignificant when compared between each other (p-value > 0.05).
14. The mean pre and post immersion dimensional differences as measured between Canine (Left) – Molar (Left) for Groups V, VI and VII were found to be 0.0320, -0.0030 and 0.0860 respectively. These differences were found to be statistically insignificant when compared between each other (p-value > 0.05).

15. The mean pre and post immersion dimensional differences as measured between Molar – Molar for Groups V, VI and VII were found to be -0.0710, -0.0200 and -0.0440 respectively. These differences were found to be statistically insignificant when compared between each other (p-value > 0.05).
16. Linear measurements between all the predetermined reference points on 3D images obtained both before and after immersion in the three disinfectants (Groups V, VI and VII) showed no statistically significant differences between the three Groups (P-value > 0.05; Insignificant).
17. All the three chemical disinfectants employed in the present study, viz. 2.4% Glutaraldehyde, 1% Sodium hypochlorite and freshly prepared Electrolyzed Oxidizing Water (EOW) showed acceptable mean $\log_{10}$ reduction values and kill rate % for antimicrobial efficacy with no significant dimensional changes between pre and post immersion PVS sample 3D image measurements.
18. Freshly prepared Electrolyzed Oxidizing Water (EOW) showed the highest mean $\log_{10}$ reduction values and 100% kill rate indicating highest antimicrobial efficacy followed by 1 % Sodium hypochlorite and 2.4 % Glutaraldehyde with all three disinfectants showing statistically similar dimensional stability.

SUMMARY

The present study was conducted to comparatively evaluate the antimicrobial efficacy of three different chemical disinfectants and their effect on the dimensional stability of polyvinyl siloxane (PVS) impressions.

The antimicrobial efficacy was determined on patient-derived PVS impressions and the dimensional stability was studied on dental model-derived PVS impressions.

To comparatively evaluate the antimicrobial efficacy, a total of 40 patient-derived PVS impressions (10 impressions X 4 Groups) were obtained and divided into four test Groups (Groups I, II, III & IV). Group I Untreated samples were treated as control. Group II samples were immersed in 2.4% glutaraldehyde, Group III samples in 1% Sodium hypochlorite and Group IV samples in freshly prepared Electrolyzed Oxidizing Water (EOW) individually for 10 minutes.

The above test group samples were subjected to microbial culture and the results were quantitatively evaluated for $\log_{10}$ count reduction values and kill rate %, and qualitatively for type and isolation frequencies of microorganisms detected.

To comparatively evaluate the dimensional stability, a total of 30 dental model-derived impressions (10 impressions X 3 Groups) were obtained and divided into three test Groups (Groups V, VI and VII). Group V specimens were immersed in 2.4 % glutaraldehyde for 10 minutes; Group VI specimens were immersed in 1 % sodium hypochlorite for 10 minutes. Group VII specimens were immersed in freshly prepared EOW for 10 minutes.

All the 30 impressions (Groups V, VI, and VII) were subjected to pre and post immersion CT scanning. All these images were reconstructed into 3D images using Mimics software and linear distances between predetermined reference points on the 3D images were measured. The predetermined points chosen were cusp tips of the right and left canine and mesiobuccal cusp tips of right and left first molar. The inter-canine, inter-molar distances and the distances between the respective right and left canines to the respective right and left molars were measured.

The results obtained from both parts of the study were statistically analyzed using one way analysis of variance (ANOVA) and student t-tests. Multiple comparisons were done by Tukey-HSD tests. All the three chemical disinfectants employed in the study showed an acceptable antimicrobial efficacy with $\log_{10}$ count reduction values around or greater than 4, the gold standard for a dental disinfectant and a kill rate % greater than 99%, with no statistically significant dimensional changes.

Among the three disinfectants studied, freshly prepared Electrolyzed Oxidizing Water (EOW) showed highest and statistically significant antimicrobial efficacy as compared to 1 % Sodium hypochlorite and 2.4 % glutaraldehyde and all three disinfectants exhibited statistically similar dimensional stability. The choice of disinfectant agents should be based on acceptable guidelines and operator preference. Electrolyzed oxidizing water is a promising option as a disinfectant for PVS impressions which needs to be investigated for further conclusive recommendations.

BIBLIOGRAPHY

1. **Abdelaziz KM, Attia A, Combe EC.** Evaluation of disinfected casts poured in Gypsum with gum arabic and calcium hydroxide additives. J Prosthet Dent 2004; 92: 27-34.
2. **Abdelaziz KM, Hassan AM, Hodges JS.** Reproducibility of Sterilized Rubber Impressions, Braz Dent J 2004 ; 15(3): 209-213.
3. **Adabo GL, Zanarotti E, Fonsec RG and Cruz CADS.** Effect of disinfectant agents on dimensional stability of elastomeric impression materials. J Prosthet Dent 1999; 81:621-4.
4. **Al-Jabrah O, Al-Shumailan Y, Al-Rashdan M,** Antimicrobial effect of 4 disinfectants on alginate, polyether, and polyvinyl siloxane impression materials, Int J Prosthodont 2007 May-Jun; 20(3):299-307.
5. **Amin WM, Muna H. Al-Alib, Sandra K. Al Tarawnehc, Tahad STH, Salehe MW, Ereifijf** N. The Effects of Disinfectants on Dimensional Accuracy and Surface Quality of Impression Materials and Gypsum Casts. J Clin Med Res 2009; 1(2):81-89.
6. **Anusavice KJ.** Science of Dental Materials, Eleventh Edition, Saunders: 2005; 225&226.
7. **Atabek D, Alacam AD, Tuzuner E, Polat S, Sipahi AB.** In-Vivo Evaluation of Impression Material Disinfection with Different Disinfectant Agents, Hacettepe Diş Hekimliği Fakültesi Dergisi, 2009;33 (2):52-59.

8. **Baumgaertel S, Palomo JM, Palomo L and Hans MG.** Reliability and accuracy of cone-beam computed tomography dental measurements. American Journal of Orthodontics and Dentofacial Orthopedics, July 2009; 136 (1): 19-25.
9. **Buck JW, Van Iersel, MW, Oetting, RD, and Hung.** In vitro fungicidal activity of acidic electrolyzed oxidizing water, Plant Dis. 2002; 86:278-281.
10. **Bustos J, Herrera R, González U, Martínez A & Catalán A.** Effect of Inmersion Desinfection with 0.5% Sodium Hypochlorite and 2% Glutaraldehyde on Alginate and Silicone: Microbiology and SEM Study, Int. J. Odontostomat 2010; 4(2):169-177.
11. **Chee WWL, Donovan TE.** Polyvinyl siloxane impression materials: A review of properties and techniques, J Prosthet dent, 1992; 68:728-732.
12. **Craig RG.** Restorative dental materials, Ninth edition. Mosby Philadelphia: 1993; 31.
13. **Donovan TE, Chee WWL.** A review of contemporary impression materials and techniques, Dent Clin N Am 2004; 48: 445-470.
14. **Drennon DG, Johnson GH, Powell GL.** The accuracy and efficacy of disinfection by spray atomization on elastomeric impressions, J Prosthet Dent. 1989 Oct; 62(4):468-75.
15. **Egusa H, Watamoto T, Matsumoto T, Abe K, Kobayashi M, Kanneda Y, Ashida S, Matusumoto T, Yatani H.** An Analysis of the persistent presence of Opportunistic Pathogens on Patient-derived dental impressions and gypsum casts**.** Int J Prosthodont 2008; 21 : 62-68.

16. **Egusa H, Watamoto T, Matsumoto T, Kobayashi M, Akashi Y Yatani H,** Clinical evaluation of the efficacy of removing microorganisms to disinfect patient-derived dental impressions, Int J Prosthodont 2008 Nov-Dec; 21(6):531-8.
17. **Estrela C, Estrela CRA, Barbin EL, Spano JCE, Marchesan MA, Pecora JD.** Mechanism of action of sodium hypochlorite. Braz Dent J (2002) 13(2): 113-117.
18. **Fabrizio KA, Cutter CN.** Stability of electrolysed oxidizing water and its efficacy against cell suspensions of salmonella typhimurium and listeria monocytogenes. J Food Prot 2003 Aug; 66(8):1379-84.
19. **Fenner DC, Burge B**, **Kayser HP and Wittenbrink MM.** The Anti-microbial Activity of Electrolyzed Oxidizing Water against Microorganisms relevant in Veterinary Medicine, J Vet Med B Infect Dis Vet Public Health. 2006 ;53(3):133-7.
20. **Firtell DN, Moore DJ, Pelleu GB.** Sterilization of Impression material for use in the surgical operation room. J Prosthet Dent. Apr' 1972; 27: 419-422.
21. **Georgescu CE, Skaug N, Patrascu I.** Cross Infection in Dentistry, Roum. Biotechnol Lett. 2002; 7 (4): 861-868.
22. **Giammanco GM, Melilli D, Rallo A , Pecorella S, Mammina C, Pizzo G.** Resistance to disinfection of a polymicrobial association contaminating the surface of elastomeric dental impressions, New Microbiologica 2009; 32: 167-172.

23. **Ivanis T, Aeivko-Babi J, Lazi B, Panduri J,** Dimensional Stability of Elastomeric Impression Materials Disinfected in a Solution of 0.5% Chlorhexidine Gluconate and Alcohol. Acta Stomatol Croat 2000; 11-14.
24. **Johansen RE, Stackhouse JA.** Dimensional changes of Elastomers during cold sterilization. J Prosthet Dent, Feb 1987; 57: 233- 236.
25. **Johnson GH, Chellis KD, Gordon GE and Lepe X.** Dimensional stability and detail reproduction of irreversible hydrocolloid and elastomeric impressions disinfected by immersion. J Prosthet Dent 1998; 79: 446-53
26. **Jones ML, Newcombe RG, Bellis H, Bottomley J.** The dimensional stability of self disinfecting alginate impressions compared to various immersion regimes. The Angle Orthodontist 1989; 60:123-128.
27. **Junevicius J, Pavilonis A, Surna A**. Transmission of Microorganisms from Dentists to Dental Laboratory Technicians through Contaminated Dental Impressions. Stomatologija, Baltic Dental and Maxillofacial Journal 2004; 6:20-23.
28. **Kamegawa M, Nakamura M, Fukui Yu, Tsutsumi S and Hojo M.** Direct 3-D morphological measurements of silicone rubber impression using micro-focus X-ray CT, Dental Materials Journal 2010; 29(1): 68–74
29. **Kollefrath R, Savary M, Schwela J.** An Evaluation of the Fit of Metal-Ceramic Restorations Made with an Autoclaved Silicone-Based Impression Material, The Journal of Contemporary Dental Practice, July 1, 2010; 11(4) : 1-9.

30. **Kronstrom MH, Johnson GH, Hompesh RW, Accuracy of a new ring-**opening metathesis elastomeric dental impression material with spray and immersion disinfection. J Prosthet Dent 2010; 103: 23-30.
31. **Kugel G, Perry RD, Ferrari M, Lalicata P.** Disinfection and Communication practices: A Survey of US Dental laboratories , JADA, June 2000; 131: 786-792.
32. **Langenwalter EM, Aquilino SA, Turner KA.** The dimensional stability of elastomeric impression materials following disinfection. J Prosthet Dent 1990; 63: 270-276.
33. **Leung RL, Schonfeld SE.** Gypsum casts as a potential source of microbial cross contamination. J Prosthet Dent Feb'1983; 49:210-211.
34. **Martin N, Martin MV, Jedynakiewicz NM.** The dimensional stability of dental impression materials following immersion in disinfecting solutions. Dental Materials 2007; 23 : 760-768.
35. **Marya CM, Shukla P, Dahiya V, Rampal N** Disinfection of dental impressions in dental colleges in India: a cause of concern. Int J Infect Control 2010; 6(1) :1-2.
36. **Matyas J, Dao N , Caputo AA, Lucatorto FM. Effects of disinfectants on dimensional accuracy of impression materials.** J Prosthet Dent 1990;64: 25-31.
37. **McCabe JF, Walls AWG.** Applied dental materials, Eighth edition**.** 1998; 148,152.

38. **Melilli D, Rallo A, Cassaro A and Pizzo G.** The effect of immersion disinfection procedures on dimensional stability of two elastomeric impression materials. Journal of Oral Science 2008; 50(4): 441-446.
39. **Merchant VA, McNeight MK, Ciborowski CG, Molinari JA.** Preliminary Investigation of a method for disinfection of dental impressions. J Prosthet Dent 1984; 52: 877-879.
40. **Minagi S, Fukushima K, Maeda N, Satomi K, Ohkawa S, Akagawa Y, Miyake Y, Suginaka H, Tsuru H.** Disinfection method for impression materials: Freedom from fear of hepatitis B and acquired immunodeficiency syndrome. J Prosthet Dent 1996; 56: 451- 454.
41. **Minagi S, Kohada A, Akagawa Y, Tsuru H.** Prevention of acquired immunodeficiency syndrome and hepatitis B. Part III: Disinfection of hydrophilic silicone rubber impression materials. J Prosthet Dent 1990; 64:463-465.
42. **Mandikos MN.** Polyvinyl Siloxane impression materials: An update on clinical use. Australian Dental Journal 1998; 43:428-434.
43. **Muller-Bolla M, Lupi-Pegurier L, Velly AM, Bolla M**. A survey of disinfection of Irreversible Hydrocolloid and Silicone Impressions in European Union Dental Schools: Epidemiologic Study. Int J Prosthodont 2004; 17: 165-171.
44. **O Brien WJ.** Dental materials and their selection, Third edition**.** Quintessence publishing: 2002; 103.

45. **Paola CL, Rocío CV, Marcela M , Milciades D and Karina CA**. Effectiveness of Electrolyzed oxidizing water for Inactivating Listeria monocytogenes in Lettuce, Universitas Scientiarum 2005; 10 (1): 97-108.
46. **Parnia F , Hafezeqoran A, Moslehifard E , Mahboub F , Nahaei Mohammadreza, Dibavar MA**, Effect of Different Disinfectants on Staphylococcus aureus and Candida albicans Transferred to Alginate and Poly Vinyl Siloxane Impression Materials, J Dent Res Dent Clin Dent Prospect 2009; 3(4):122-125.
47. **Pichler W, Windisch G, Schaffler G, Heidari N , Dorr K, Grechenig W** . Computer-assisted 3-dimensional Anthropometry of the Scaphoid. Orthopedics 2010 Feb; 33(2):85-8.
48. **Powell GL, Runnells RD, Saxan BA, Whisenant BK**. The presence and identification of organisms transmitted to dental laboratories. J Prosthet Dent 1990; 64:235-237.
49. **Puttaiah R, Griggs JA, Kanabar J and Coon D.** Effects of an Immersion Disinfectant and a Surface Disinfectant on Three Elastomeric Impression Materials J Dent Res 2003; 82.
50. **Rampal N, Pawah S, Kaushik P.** Infection Control in Prosthodontics. JOHCD January 2010; 4(1): 7-11.
51. **Ray KC and Fuller ML.** Isolation of Mycobacterium from dental impression material. J. Prosthet. Dent Jan.-Feb, 1963; 93-94.
52. **Rueggeberg FA, Beall FE, Kelly MT, Schuster GS.** Sodium hypochlorite disinfection of irreversible hydrocolloid impression material. J Prosthet Dent 1992: 67: 628-630.

53. **Runnels RR.** An overview of infection control in dental practice. J Prosthet Dent 1988; 59:625-629.
54. **Samaranayake LP, Hunjan M, Jennings KJ.** Carriage of Oral flora on irreversible hydrocolloid and elastomeric impression materials. J Prosthet Dent 1991; 65: 244- 249.
55. **Shillingburg HT, Hobo S, Whitsett LD, Jacobi R, Brackett SE.** Fundamentals of Fixed Prosthodontics. Third edition. Quintessence publishing: 1997; 281.
56. **Silva SMLMD, Salvador MCG**. Effect of the disinfection technique on the linear dimensional stability of dental impression materials. J Appl Oral Sci 2004; 12(3): 244-9.
57. **Stober T, Johnson GH, Schmitter M.** Accuracy of the newly formulated vinyl siloxanether elastomeric impression material. J Prosthet Dent 2010; 103: 228-239.
58. **Taylor RL, Wright PS, Maryan C.** Disinfection procedures: their effect on the dimensional accuracy and surface quality of irreversible hydrocolloid impression materials and gypsum casts. Dental Materials 2002; 18: 103-110.
59. **Tullner JB, Commette J, Moon PC.** Linear dimensional changes in dental impressions after immersion in disinfectant solutions. J Prosthet Dent 1988; 60:725-728.
60. **Valderhaug J, Odont, Floystrand F.** Dimensional stability of elastomeric impression materials in custom made and stock trays. J Prosthet Dent 1984; 52: 514-517.

61. **Venkitanarayanan KS, Ezeike GO, Hung Y and Doyle MP.** Efficacy of Electrolyzed Oxidizing Water for Inactivating Escherichia coli O157:H7, Salmonella enteritidis, and Listeria monocytogenes, Applied and Environmnental microbiology Sept. 1999; 4276–4279.
62. **Wadhwani C.P.K., Johnson GH, Lepe X, Raigrodski A.J,** Accuracy of newly formulated fast-setting elastomeric impression materials, J Prosthet Dent 2005; 93: 530-9.
63. **Wu G, Yu X, Gu Z.** Ultrasonically nebulised electrolysed oxidizing water: a promising new infection control programme for impressions, metals and gypsum casts used in dental hospitals. Journal of Hospital Infection 2008; 68: 348-354.